Metallic nanoparticles, used since antiquity to impart intense and vibrant color into materials, have more recently become a central tool in the nanoscale manipulation of light across a range of chemical sciences and engineering applications. This interest has led to a virtual explosion of new types of metal-based nanoparticles and nanostructures of various shapes and compositions, and has given rise to new strategies to harvest, control, and manipulate light based on these structures and their properties. By assembling metallic nanoparticles into useful building blocks, a striking parallel between the plasmons of these structures and wave functions of simple quantum systems is universally observed. Clusters of metallic nanoparticles behave like coupled oscillators or antennas, introducing effects characteristic of systems as diverse as radio frequency transmitters and coupled pendulums into light-driven nanoscale structures. Their unique light-controlling properties can be put to use in a multitude of ways: for detecting single molecules and following chemical reactions, for generation of hot electrons for color-specific photodetection and photocatalysis, and most recently, for high-efficiency solar steam generation poised to tackle our planet’s energy and sustainability challenges.


**Session #** 20  |  **Abstract #** 20-1  |  **Awards**
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**Session Title** | Pittcon Heritage Award |  
**Abstract Title** | Presentation of the 2015 Pittcon Heritage Award |  
**Primary Author** | A Blaine Bowman |  
**Author** | Illumina |  
**Co-Author(s)** |  
**Abstract Text** | To be presented at Pittcon 2015 by Carsten Reinhart, Chemical Heritage Foundation President |  
**Date:** | Sunday, March 08, 2015 - Afternoon |  
**Time:** | 04:30 PM |  
**Room:** | Great Hall A |  
**Keywords:** | Education |  
**Application Code:** | Other |  
**Methodology Code:** | Education/Teaching |
Microcystins are formed by cyanobacteria during algal blooms, and are found all around the world. These liver toxins can contaminate drinking water supplies, and can be fatal for many species of animals, as well as humans. Data will be presented showing levels of some of the more widely-reported compounds, such as Microcystin-LR, but also the identification of different variants by interpretation of accurate mass fragmentation spectra using LC-QTOF.
Comprehensive two-dimensional gas chromatography (GCxGC) used in combination with high-resolution mass spectrometry (HRMS) is a powerful tool for the analysis of complex mixtures. In this work, we analyzed a number of environmental samples by using GCxGC in combination with a new high-resolution time-of-flight (TOF) mass spectrometer. A composite mass spectrum for each sample was created by summing the mass spectra for all components in the GCxGC/HRTOFMS analysis. Afterwards, the Kendrick mass defect plots produced from these spectra facilitated the rapid identification of families of compounds based on their substituents which in turn helped guide the analysis of the chromatographic data. Additionally, tentative identification of specific contaminants was aided by database searches and elemental composition determinations from the accurate-mass data. The details of these results will be reported for this presentation.

Keywords: Environmental Analysis, Gas Chromatography, Time of Flight MS, Trace Analysis
Application Code: Environmental
Methodology Code: Other
Analysis of commercial honey from different parts of the US was carried out using a simple sample preparation procedure. Raw honey samples were dissolved in water and directly injected into a liquid chromatography-quadrupole time-of-flight mass spectrometer (LC/Q-TOF-MS). A complex profile of compounds was obtained after processing the mass spectral data. Identifications of a few natural compounds were made by accurate mass measurements. Moreover MS-MS experiments were performed to elucidate structures of the found compounds.

Maple syrup samples from different regions were also analyzed and compared. Because of both grade and region, verification of maple syrup is difficult. Similarly to honey, analysis of maple syrup shows a complex profile of compounds. Direct analysis of real and imitation maple syrup easily distinguishes the two. Ratios of glucose to fructose can be used to distinguish the amount of natural sugar to process sugar in both types of maple syrup. Both adulteration and classification of the syrup by grade and region is more complex and requires data mining and multivariate statistical approach. This work examines the chemical profiling of syrups from different regions and grades. Identification of marker compounds for real maple syrup will be made by LC/Q-TOF-MS and compared.

Keywords: Environmental Analysis, Food Contaminants, Liquid Chromatography/Mass Spectroscopy, Natural Pr
Application Code: Food Identification
Methodology Code: Liquid Chromatography/Mass Spectrometry
Current European Union regulations permit the use of GC/MS-MS and bioassay techniques only in feed and food samples. In this talk we will present a comparison of results obtained with official GC/HRMS methods, with a GC/HRMS TRACE GC 2000, Thermo Finnigan (Thermo Fisher Scientific), coupled with a MAT 95 XP Mass Spectrometer, and with a commercial GC/MS-MS Agilent 7000 Triple Quadrupole Mass Spectrometer interfaced to an Agilent 7890 GC (Palo Alto, CA, USA), for the dioxins analysis in typical air and soil environmental samples. Most of the samples analyzed had congeners below LOD as they were real, everyday, environmental samples. A correlation between PCDD/F soil and air samples concentrations, expressed as WHO-TEQ, obtained by GC/MS-MS and GC/HRMS, despite the low PCDD/F concentrations, shows that data are in a good agreement.

A new monitoring strategy for dioxins in environmental samples could be proposed with new MS/MS instrumentation available. This would be a realistic, lower cost, higher throughput alternative to the GC/HRMS method at least as a screening method to classify a sample as compliant or suspected to be non-compliant.

Keywords: Analysis, Environmental/Air, Environmental/Soils, GC-MS
Application Code: Environmental
Methodology Code: Mass Spectrometry
This abstract reports the identification of various surfactants in flowback and produced water samples from hydraulic fracturing resulting from natural gas production. The method uses an application of the Kendrick mass defect and liquid chromatography/quadrupole-time-of-flight mass spectrometry, including ion mobility. The Kendrick mass defect differentiates the various adducts of the ethoxylates, in particular, the proton, ammonium, and sodium adducts in both singly- and doubly-charged forms. ICP/MS is also applied to the flowback waters to identify constituents used in making up the cocktail for injection. The importance of the various identified surfactants will be discussed as well as procedures for removal of these compounds from flowback and produced water from hydraulic fracturing.

Keywords: Energy, Environmental/Water, Mass Spectrometry
Application Code: Environmental
Methodology Code: Mass Spectrometry
There is increasing interest in the use of nanoparticles (NPs) for biomedical applications. In particular, nanobiophotonic approaches using fluorescence offers the potential of high sensitivity and selectivity in applications such as fluorescence-based bioassays and intracellular sensing. In this presentation, we focus primarily on the use of fluorescent silica NPs for these applications. We summarise the main synthetic approaches namely the Stöber and microemulsion processes and, in this context, we deal with issues in relation to both covalent and physical incorporation of different types of dyes in the particles. The important issue of NP functionalisation for conjugation to biomolecules is discussed and strategies for minimising non-specific binding are highlighted. We give examples of the use of silica NPs as fluorescent labels for bioassays and as carriers for analyte-specific dyes for intracellular sensing applications, in particular for optical oxygen and pH sensing. The use of the NPs for cell staining is also discussed in the context of cancer diagnostics. We conclude with some perspectives for the future in this area.

Acknowledgements
This work was supported by Science Foundation Ireland (Grant No 10/CE/B1821) and by the Higher Education Authority (HEA) grant to the Targeted Therapeutics and Theranostics (T3) programme in DCU under PRTLI IV.

Keywords: Fluorescence, Immunoassay, Nanotechnology, Sensors
Application Code: Biomedical
Methodology Code: Sensors
Nanotechnology has emerged as enabling platform for using nanofabrication and nanoparticle formulations to improve the detection of chemical and biological processes. In particular, silica nanoparticles provide a versatile platform to boost the photostability and fluorescence quantum yields of near infrared (NIR) fluorescent dyes. We demonstrate the development of NIR fluorescent silica nanoparticles, which enhanced the brightness of NIR dyes by orders of magnitude. Coupled with exceptional photostability, these nanoparticles are useful for longitudinal assessment of the same sample or cell population without loss of fluorescence signal, ushering a new approach for monitoring dynamic processes in cells and living organisms.

Keywords: Fluorescence, Imaging, Nanotechnology, Near Infrared
Application Code: Biomedical
Methodology Code: Near Infrared
A practical method for bacterial separation remains a hindrance for the successful deployment of rapid detection methods from complex samples. We have developed core-shell nanoparticles containing 30% cobalt (w/w) in the final nanoparticle which have much shorter separation times than traditional iron oxide nanoparticles. The particles were bound to T7 bacteriophages in order to recognize and specifically bind to \textit{E. coli} cells. For comparison, the particles were directly compared to antibodies on both the magnetic nanoparticles as well as traditional micron-scale particles. The capture efficiencies of antibodies and bacteriophages on nanoparticles and microparticles for the separation of \textit{E. coli} K12 were determined. The results suggest that improvements can be made to magnetic separation by using phage for capture and by the use of nano-scale particles.

A paperfluidic device was constructed to detect the separated \textit{E. coli}. Here we report the development of a simple and sensitive antibody and Ru(bpy)$_3$$_2^{2+}$-doped silica nanoparticle-labeled lateral flow assay which achieves low limit of detection. Following concentration, the nanoparticles and bacteria were incubated to allow completion of the bacteriophage infection cycle. The increase in free phage titer was then quantified fluorescently.

The 30 nm spherical Ru(bpy)$_3$$_2^{2+}$-doped silica nanoparticles were prepared in aqueous medium by a novel method recently reported. The nanoparticles were modified anti-T7 antibodies. The experimental results showed that the lateral flow fluorescent assay developed was more sensitive compared with the traditional colloidal gold test strips. The limit of detection for the fluorescent lateral flow assay developed is approximately $10^{6}$ PFU fmols as compared to approximately $10^{8}$ PFU for the colloidal gold. This correlated with a detection limit of approximately $10^{3}$ CFU/mL of \textit{E. coli}.

Keywords: Agricultural, Biosensors, Food Contaminants, Food Safety

Application Code: Food Safety

Methodology Code: Sensors
A series of silica-based near-infrared fluorescent (NIRF) nanomaterials have been developed using new approaches. The distinct features of these NIRF nanomaterials are 1) highly intense and photostable fluorescence signals, 2) tunable fluorescence wavelengths, and 3) low toxicity. Several metallic-based sandwich nanostructures were developed with various shapes and sizes for further enhanced fluorescence. The geometric, compositional, and dimensional effects of these NIRF materials on their fluorescence properties were studied, including fluorescence quantum yield, lifetime and wavelengths. These NIRFs were applied to biological applications including sensitive detection and imaging of trace analytes, and photothermal therapies. Nanomaterial-based photothermal therapy has shown great potential for efficient cancer treatment. The developed nanohybrids can generate significant amount of heat upon irradiation in the near infrared (NIR) region for inducing thermal cell death. The nanohybrids showed excellent in vitro biocompatibility as a promising hyperthermia agent.

Keywords: Fluorescence, Modified Silica, Nanotechnology, Near Infrared

Application Code: Nanotechnology

Methodology Code: Near Infrared
Fluorescent silica nanoparticles can be made using almost any kind of fluorophores that can be modified to have suitable functional moiety for covalent binding to silicates used in nanoparticle synthesis. Chemically stable visible fluorophores such as fluorescein is often utilized for this purpose. The NIR spectral region (650-900 nm) offers significant advantages among them lower background interference especially in biological samples. However NIR dyes have relatively lower fluorescent quantum yield as compared to visible fluorophores. Additional disadvantage of using NIR dyes is their relatively high sensitivity to microenvironmental changes such as solvent polarity or quenching. Self quenching may become a problem due to energy transfer for carbocyanines that are at high concentration in the silica nanoparticle. Self quenching can be significantly reduced by using dyes that have large Stokes’ shift. This can be achieved by substituting meso position halogens in the NIR fluorescent carbocyanine dye with a linker containing amino moiety. This substitution can also serve as a linker to covalently attach the dye molecule to the nanoparticle backbone. Additional disadvantage of NIR dyes is their relative sensitivity to harsh chemical conditions present during silica nanoparticle synthesis. During the studies presented here we developed silica nanoparticles containing different covalently incorporated visible and NIR dyes. One application of these particles is for bright fluorescent labels to be used in immunochemistry, flow cytometry, etc. Covalently bound silica nanoparticles can be used as biomolecular labels after activation with aminoreactive surface moieties suitable for biomolecule labeling. Other biological and bioanalytical applications of fluorescence silica nanoparticles presented includes phagocytosis and related applications. Reverse micelle synthesis approach to this application is ideal due to easy control of particle size.
Nitric oxide has been implicated in a number of physiological processes, including vasodilation, neurotransmission, sepsis, and wound healing. As a result, the quantification of nitric oxide and its analogs in vivo may lead to improved diagnosis and treatment. Our lab has previously described the fabrication of a microfluidic nitric oxide sensor platform capable of sensitive and selective detection of nitric oxide in biological fluids. Samples volume requirements (1.0 milliliters) circumvented using this device for wound fluid analysis. Furthermore, the first generation device only allowed for the detection of nitric oxide. In this presentation we will describe an improved microfluidic sensor that requires only 50 microliters of sample and allows for measurement of nitric oxide, nitrite, and S-nitrosothiols.

**Keywords:** Bioanalytical, Electrochemistry, Lab-on-a-Chip/Microfluidics, Microelectrode

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
One of the predominant challenges in systems-wide analyses is the broad-scale characterization of the molecular inventory in cells, tissues, and biological fluids. Advances in computational systems biology rely heavily on the experimental capacity to make panomics measurements, i.e. integrated metabolomics, proteomics, lipidomics, glycomics, etc., accompanied with fast minimal sample preparation, fast measurements, high concentration dynamic range, low limits of detection, and high selectivity. This confluence of figures-of-merit place demanding challenges on analytical platforms for such analyses. Ion mobility-mass spectrometry (IM-MS) provides rapid (ms) gas-phase electrophoretic separations on the basis of molecular structure and is well suited for integration with rapid (us) mass spectrometry detection techniques. Furthermore, the timescales of this multi-dimensional separation are well suited for combination with fast condensed-phase separations such as GC, SFC, and UPLC (min) for enhanced separation selectivity as the sample complexity becomes ever more challenging. This report will describe recent advances in IM-MS panomics measurement strategies in the analyses of complex biological samples of interest in systems, synthetic, and chemical biology. New advances in bioinformatics and biostatistics will also be described to approach biological queries from an unbiased and untargeted perspective and to quickly mine the data gathered to provide targeted and actionable information. Elucidation of biomolecular signatures specific to diabetic wound healing and in synthetic biological studies to decipher the prevailing biology in a variety of diseases and drug mode-of-action will be presented.
Sol-gel processing is widely used to create low-k materials, thermal insulation, and stationary phases in the separation sciences. Xerogels, porous sol-gel derived materials formed by solvent evaporation at or near ambient conditions, are attractive platforms for chemical sensor development. We have devoted significant time and effort to elucidate the chemistry within hybrid silica-based xerogels as a means to intelligently guide materials development for use in areas ranging from chemical sensors to anti-fouling coatings. The speaker will summarize their research group’s efforts to develop sensors for time-dependent cytokine and growth factor detection and imaging in model systems.
Bacterial contamination of chronic wounds has long been a major concern—especially for those involved in the management of diabetic foot disease where delays in treatment can all too easily lead to life threatening complications. While there have been considerable advances in the development of wound dressings that aim to minimise bacterial contamination and aid the healing process, the majority tend to be inherently passive and possess little or no diagnostic capability.

Early identification of infection is imperative in the management of the diabetic foot in preventing limb threatening events, the need for hospitalisation and subsequent amputation but it is possible and, indeed probable, that individuals will only seek treatment once gross symptoms appear and an infection has taken a firm hold. There is a pressing need for more advanced materials that could not only provide the clinician with a detailed picture of the wound dynamics at the time of consultation, but which would periodically monitor the wound once they leave the hospital.

Conductive films capable of monitoring chemical changes within the wound fluid open up the possibility of remote monitoring of the wound thereby allowing much earlier referral and hence proffer a more effective treatment and a more positive outcome. The design of mechanically flexible composite film structures, their activation for electrochemical sensing and subsequent response to a range of wound biomarkers are described. Their assembly within conventional bandage as an integrated unit is demonstrated and their adoption for clinical use is discussed.

Keywords: Bioanalytical, Electrochemistry, Electrodes, Sensors
Application Code: Biomedical
Methodology Code: Electrochemistry
Chronic wounds such as diabetic ulcers, venous leg ulcers and pressure sores are very difficult to heal and can last for months to years in spite of the numerous treatments currently available. Various wound biomarkers have been investigated as possible predictors of this healing impairment. Our preliminary human studies of wound fluid nitric oxide levels have suggested a correlation between wound progression (healing) or worsening and threshold levels of nitric oxide activity. We analyzed nitric oxide metabolites (nitrate and nitrite, NOx) in human wound fluids and correlated these markers with wound healing status (progressing or worsening) based on patient’s wound history. Samples were collected from patients with wounds of various etiologies and analyzed for NOx. A laboratory method was developed to analyze NOx which can detect at least 5 uM in samples as small as 10 ul. A nitrate-free sample collection device was identified to match the sensitivity of this new assay (most “nitrate-free” products tested contained nitrate levels higher than this detection limit when extracted in such a small volume). Data analysis from 50 wounds showed that NOx allowed to discriminate between worsening and progressing wounds and suggested that a two cut-point diagnostic test using NOx is better than a single cut-point test to identify progressing from worsening wounds. The method developed here can be used in the future to monitor NOx levels in wounds producing enough exudate for its prognostic value or to monitor the response to a therapy. This could provide a useful tool to help guide treatment in wound care. Additional work is needed to get longitudinal data and a larger number of wound patients in each of the various chronic wound categories.
Despite its effectiveness at removing background intereferrent signal from mass spectral analyses, FAIMS has yet to achieve wide-spread use for a number of factors. The first, and foremost, complaint is limited sensitivity. It is common to lose approximately ninety percent of incoming ion flux when FAIMS is implemented. Although the resulting signal-to-noise can be improved versus analysis without FAIMS, the loss in absolute ion signal does not allow for lower limits of quantitation in most cases. Second, the amount of time required for FAIMS separation, often between 50ms and 100ms, limits the number of data points available across a chromatographic peak. Third, a mixture of nitrogen and helium is generally used as FAIMS gas. While it has been shown that helium in the transport gas offers beneficial results, the reduced worldwide availability of helium, and its resulting cost, is a mark against FAIMS. Finally, FAIMS analysis has been mostly limited to liquid flow rates at microliters per minute and above. This is due to the desolvation gas flowing into the source region deflecting the electrospray plume away from the entrance of the device. This is overcome when using ionization sources that are aided by a sheath gas to push through the countercurrent desolvation gas. However, nanospray generation in front of the entrance plate has been difficult to date. Here, we will discuss modifications to a set of cylindrical FAIMS electrodes that address each of the previous factors limiting FAIMS analysis. The resulting nanospray friendly instrument is capable of transmission efficiencies greater than fifty percent with residence times lower than 20ms without using helium or dopants.
Glycosaminoglycans (GAGs) are an important class of carbohydrates that are involved in a number of key biological processes. Their biosynthesis occurs as a number of sequential enzymatic processes in the Golgi apparatus. Because these are non-template events, GAGs are produced as a heterogeneous mixture of molecular sizes and compositions. Their analysis is challenging both because of the complexity of the mixture of structures that are present in a sample as well as the lability of their chemical features, specifically sulfation. There have been many recent advances in "sequencing" purified GAG oligomers by tandem mass spectrometry. To examine mixtures of GAGs, there needs to be some separation of the components prior to MS analysis. On-line HPLC, which works well for peptides, does not work so well with GAGs, as they are highly ionic, and not well separated by conventional reverse phase separations. As an alternative to solution-phase separations, we are developing gas-phase separation of GAGs based on differential ion mobility. We find FAIMS to provide excellent resolving characteristics for GAG oligomers, ionized by electrospray ionization in negative ion mode. FAIMS can resolve fine structural differences, such as the epimeric sugar residues, glucuronic acid from iduronic acid, in tetrameric oligomers. This presentation will describe the recent progress in applying FAIMS to the analysis of GAGs using FTICR mass spectrometry.

Keywords: Carbohydrates, Electrospray, Ion Cyclotron Resonance, Method Development
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
The design of a DMS cell is flexible and can be tailored to the performance requirements of targeted applications. The focus of this presentation will be on the specific design elements of a DMS/MS system, how they affect performance, and how they can be balanced to achieve the appropriate performance trade-offs. Particular emphasis will be given to how factors such as DMS gap height and residence time affect key figures of merit, including resolution, peak capacity, transmission, and analysis speed. Examples of separations will be provided with a range of different mobility cell dimensions and geometries. In addition, modeling results will be provided to describe the development of simulators of ion motion, whose ultimate value can be envisioned for aiding in DMS instrument design optimization and creating reference libraries of differential mobility for a large number of chemical species for chemical identification purposes. In particular, the use of simulators to improve our understanding of fringing field effects and temperature gradients will be discussed.

Keywords: Instrumentation, Mass Spectrometry
Application Code: General Interest
Methodology Code: Other
An improved miniature FAIMS system configuration for high-resolution extreme field separations will be described. Critical detector performance metrics (relating selectivity and sensitivity) will be assessed by case study of Volatile Organic Compounds (VOCs) and other low molecular weight compounds.
A unique feature of differential ion mobility spectrometry (FAIMS) is a strong dependence of separation performance on the buffer gas composition. In that aspect, FAIMS is distinct from conventional IMS but resembles liquid chromatography, where the species resolution is sensitive to the chemistry of mobile and stationary phases and is often optimized by their tailoring. However, FAIMS differs from chromatography in that the choice of gas also greatly affects the overall separation peak capacity. This talk will discuss our systematic effort to maximize the FAIMS resolving power and resolution of specific targets by rational gas formulation. We shall focus on the advantages of buffers rich in helium and particularly hydrogen, especially for analyses of protein conformers in comparison with smaller ions such as peptides, lipids, and amino acid isotopomers. The limitations of approach for larger macromolecules imposed by the peak broadening due to conformational diversity will be explored.

Keywords: Electrospray, Mass Spectrometry, Protein, Separation Sciences
Application Code: Bioanalytical
Methodology Code: Separation Sciences
Environmental research continues to expand beyond traditional, regulated contaminants to emerging contaminants, such as pharmaceuticals, perfluorinated compounds, nanomaterials, flame retardants, drinking water disinfection by-products (DBPs), hormones, siloxanes, UV filters, algal toxins, naphthenic acids, benzotriazoles, artificial sweeteners, antimony, perchlorate, musks, and prions. These are now frequently being found in water samples, including rivers, lakes, ground water, and drinking water. Moreover, understanding their fate and transport in the environment and in wastewater/drinking water treatment is vitally important, and as such, one of the major trends continues to be in identifying their transformation products. Because environmental samples are inherently complex mixtures with trace-level contaminants, the development of sensitive and modern analytical tools has been key for their identification and measurement. This presentation will provide an overview of emerging contaminants and the modern tools used to measure them. This will include a discussion of high resolution mass spectrometry (MS), liquid chromatography (LC)/MS/MS, gas chromatography (GC)xGC-MS, direct sampling ionization-MS techniques, NMR spectroscopy, and halogen-specific total organic halogen (TOX) analysis, as well as current extraction technologies.

Keywords: Environmental/Water, Mass Spectrometry
Application Code: Environmental
Methodology Code: Mass Spectrometry
Micro-scale analytical systems positively influence the cost, availability and range of applications of environmental analysis. Highly portable, energy efficient field analysis systems, monitoring networks, and remote/autonomous devices can all be realized through miniaturization.

This work presents the design, fabrication and application of the components of an integrated field-portable analysis device for water quality monitoring. Micro-fabricated components include filters, mixers, reagent reservoirs, and detection zones. Detection is accomplished using stand-alone, low-cost hardware and the on-board optical hardware of a smartphone. The presentation includes an overview of design elements, fabrication methods, and integrated device models.

An application to be discussed is analysis of environmental water for evidence of hydraulic fracturing (fracking) contaminants. While fracking has made shale gas accessible and economical, large quantities of wastewater are produced over the lifespan of a well: drilling and fracturing a Marcellus shale well requires 14,000-17,000 m3 of water. Within one year of well completion, 10-25% of the water returns to the surface as a concentrated brine. 55-80% of this returned water is reused for other wells, leaving a substantial amount of wastewater. New approaches are needed to assess and ensure compliance and safety as these valuable natural resources are utilized. Constituents such as bromide, strontium, and barium are characteristic of the wastewater generated by Marcellus shale gas extraction, and are of particular interest as early indicators of wastewater leaks and for tracking the movement of produced waters.

Our progress toward the ultimate goal of a versatile, portable, simple, stable, adaptable, low-cost monitoring platform will be highlighted.

Keywords: Environmental, Environmental/Water, Fuels\Energy\Petrochemical, Lab-on-a-Chip/Microfluidics
Application Code: Environmental
Methodology Code: Microfluidics/Lab-on-a-Chip
In recent years, chemicals of emerging concern have been associated with adverse effects observed in aquatic organisms throughout the world. These include feminization of male fishes, reduced reproductive success, immunosuppression and behavior changes. However, the specific causes and sources associated with these responses are often difficult to identify. Many of these chemicals, such as synthetic hormones, pharmaceuticals, personal care products were produced to have biological effects. They act through interaction with hormone receptors, often involving complex feedback mechanisms and hence may elicit nonlinear dose responses. Aquatic organisms are exposed to complex mixtures of these as well as legacy contaminants that may also have endocrine-disrupting effects. These complex mixtures may contain compounds with additive, synergistic or antagonistic activity and can have biological effects at concentrations below or close to the current level of detection. Hence, biological effects monitoring is increasingly used to identify potential problems. Bioindicators, such as intersex (testicular oocytes), vitellogenin (a yolk precursor) in the blood of male fishes, altered plasma hormone concentrations, increased susceptibility to infectious disease, microscopic changes in gonads and endocrine glands, and changes in gene expression profiles of wild fishes have been developed and used in numerous monitoring and assessment projects. In addition, extracts from discrete and time integrated water samples can be screened for hormone (estrogenic, androgenic, thyroid, corticosteroid) activity using in vitro cell-based assays such as the BLYES (bioluminescent yeast estrogen screen), transgenic zebrafish embryos or nuclear translocation assays. These methods are considerably cheaper and quicker than comprehensive chemical analyses and can better focus the choice of analytes in water, sediment or fish tissue.

Keywords: Bioanalytical, Biological Samples, Environmental/Biological Samples, Environmental/Water
Endocrine disrupting contaminants that enter the water system through ineffective wastewater treatment and as run-off, impair reproduction, interfere with development, and are associated with obesity and cancer in humans. Fish are indicators of environmental exposure that is hazardous to humans, and chemical biomarkers in fish are correlated to early stages of dysfunction. Circulating steroids are critical biomarkers of endocrine disruption because they are a direct measurement of hormonal imbalance initiated by endocrine disrupting contaminants. Capillary electrophoresis is utilized to rapidly quantify steroid hormones circulating in only 5 microliters of plasma in order to identify endocrine disrupting chemicals that elicit a physiological response. Multiple steroidal compounds are captured, concentrated and assayed using a high-throughput label-free electrophoresis separation based on pH mediated stacking. With this new technology, nanomolar detection of steroid hormones is achieved with UV-visible absorbance. When the microscale method is used to profile steroids in individuals, the change in steroid levels is easier to quantify and observe. The technology is utilized to demonstrate how chemicals and chemical mixtures interfere with interrelated steroid pathways. The effects of solvents used in the literature to solubilize and deliver chemicals are also evaluated.

This work is supported through NIH R21ES023575-01.
High sensitivity, high mass accuracy mass spectrometry has transformed the way we examine biological and biomedical questions. With modern analytical tools, proteome-wide studies of protein post-translational modification and single amino acid polymorphisms (SAAPs) are enabled. Expression levels are accurately quantified by single reaction monitoring (SRM). Furthermore, with the recent integration of the ENCODE data into our proteomic workflow, we can identify the unknown human proteins at an accelerated pace. By use of databases that contain predicted sequences of known and novel proteoforms, we identified dozens of SAAPs in proteins derived from glioma stem cells that may be associated with glioma pathology, and novel alternative spliceforms derived from fusions between known and previously unknown exons. The identification of an overexpressed metabolic protein, BCAT2 T186R, has opened up studies of a target never associated with glioma pathology. Integration of RNA-Seq data and proteomics has allowed us to determine the roles of SAAPs and novel fusion proteins in GSC pathobiology. Bioinformatic searches of ENCODE data translated into a searchable database for proteomics (proteoENCODE db) yielded eighty-one chimeric peptides in GSCs. These results will allow further studies of proteins' role in pathogenesis, tumor recurrence, and resistance to chemotherapy and radiation. The next step is to validate and quantify their expression by SRM across our biobanked cell lines, at baseline conditions and following standard-of-care treatments. We expect that among the newly identified proteins, new therapeutic targets and biomarkers will be established. Our results are enabled by modern mass spectrometric methods and the development of databases that include the complexities of protein features. Our results serve to demonstrate how improved analytical methods have translated into increased ability to produce breakthrough discoveries in cancer research.

Keywords: Bioinformatics, Informatics, Mass Spectrometry, Proteomics
Application Code: Biomedical
Methodology Code: Mass Spectrometry
Protein footprinting coupled with mass spectrometry has emerged in recent years as a valuable tool to study protein structure. Several footprinting techniques, such as hydrogen deuterium exchange and oxidative labeling, have been successfully used to identify protein-protein and protein-ligand interactions in varying protein systems. To date, these footprinting methods have been performed on relatively pure proteins \textit{in vitro}. The development of an \textit{in vivo} footprinting method would provide a powerful platform to study proteins in their native cellular environment. The coupling of footprinting with high resolution mass spectrometry allows for data to be analyzed on the amino acid residue-level. We have developed an \textit{in vivo} oxidative labeling method, based on the footprinting method fast photochemical oxidation of proteins (FPOP). Oxidative labeling is advantageous for \textit{in vivo} labeling owing to its irreversible nature, which allows for purification of the proteins of interest from the cell membrane prior to MS analysis. Several parameters were optimized for the development of \textit{in vivo} FPOP, including hydrogen peroxide concentration and quenching conditions. Mass spectrometry on digested cell lysates demonstrate that several proteins within the cell are oxidatively modified. A comparison of the extent of oxidative modification to regions of solvent accessibility of several proteins whose structure has been solved by high resolution methods revealed that \textit{in vivo} FPOP successfully characterizes solvent accessibility. These data demonstrate the feasibility of \textit{in vivo} FPOP as a tool to analyze protein structure within cells.

Keywords: Bioanalytical, Biological Samples, Mass Spectrometry, Protein
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
This presentation will provide several examples of how we re-discover, objectively learn, and generate new hypotheses using ultrahigh specificity technology. For example, it is often thought that a Western Blot is a gold standard for the specific detection of a protein and that quantitative Western blotting provides quantitative information. Mass spectrometry is inherently specific and can be quantitative over 5 orders of magnitude. Moreover, it provides us with untargeted yet specific data enabling new discoveries and hypotheses to be generated. Examples from several different areas of biology will be presented including ovarian cancer and lignin biosynthesis.
Proteomics allows the simultaneous detection of thousands of proteins and can give dynamic information about underlying mechanisms of disease. Using a transgenic mouse model, our group has gained insight about the proteome of Alzheimer’s disease in systemic tissues. In order to follow protein changes with disease progression and from many biological replicates, has required the development of novel sample multiplexing methods. These sample multiplexing methods rely on precursor isotopic labeling and isobaric tagging methods and take advantage of mass spectrometry to keep track of sample origin and relative sample quantity. Here we will briefly present these methods and discuss how they have been used to understand the complexity of Alzheimer’s disease.
Measuring diagnostic panels of multiple proteins promises future personalized early detection and therapy monitoring of cancer. Levels of biomarker proteins in patient serum can provide a continually updated record of disease status. Research in electrochemical detection of proteins has produced exquisitely sensitive approaches. Most utilize ELISA-like sandwich immunoassays incorporating various aspects of nanotechnology. Several of these ultrasensitive methodologies have been extended to microfluidic multiplexed protein detection. To achieve clinical or point-of-care (POC) use, simplicity and low cost are essential. In multiplexed microfluidic immunoassays, required reagent additions and washing steps pose a significant problem calling for creative engineering. We will discuss two microfluidic systems utilizing strategies to solve these problems to detect 4 protein biomarkers in serum. The first involves a sample-reagent delivery system feeding an ECL detection chip built with carbon nanotube forests in microwells, and using RuBPY-loaded antibody-coated silica nanoparticles for detection. The second example involves on-line capture of analyte proteins on antibody-enzyme-coated magnetic beads with detection on an 8-electrode antibody decorated chip. Applications to development of diagnostic protein panels for prostate cancer and oral mucositis during radiation therapy and validation with patients serum samples will be discussed.
Microfluidic paper-based analytical devices (mPADs) have gained attention as an alternative format for microfluidic analysis because of their low cost, flexibility of analysis methods, and wide range of potential applications. While mPADs have utilized a variety of detection methods, colorimetric reactions have been the most common. However, electrochemical detection with mPADs (ePADs) provides an attractive alternative to colorimetric methods because it is more sensitive, selective, and can access a wide range of analytes. Here recent research from our group on portable ePAD development and chemistry will be discussed for four different ePAD configurations with an emphasis on two projects: detection of pollutants in water, and analysis of enzymatic reactions specific to microbe detection. The first ePAD design incorporates novel carbon paste microelectrodes to detect organic pollutants such as pesticides. Second, an ePAD utilizing a simplified method for incorporating carbon electrodes has been developed and used to measure pesticide contamination marker, p-nitrophenol, in ground water. By coupling this system with a built in QR code, it is possible to provide simple and rapid assessment of pollutant levels. Third, a flow injection analysis-like ePAD system will be presented. This ePAD sandwiches Au microwire electrodes between layers of paper to create a flow through analyzer. The physical shape of the pumping region allows for steady-state flow with multiple sample injections. Finally, carbon paste ePAD detection of enzymes associated with E. Coli and Salmonella will be presented as a first step towards analysis of bacterial loads in food and water.

Keywords: Aerosols/Particulates, Electrochemistry, Environmental Analysis, Lab-on-a-Chip/Microfluidics
Application Code: Environmental
Methodology Code: Electrochemistry
### Abstract Text

The ability to provide real-time information about an individual’s response to therapeutics has the potential to revolutionize healthcare by offering personalized treatment. Drugs with narrow therapeutic windows become toxic or ineffective if over- or under-dosed thus reducing efficacy. Maintaining the most efficient dosage, personalized to the patient, can maximize efficacy of treatment and ultimately patient outcome. Current methodologies for monitoring therapeutics are cumbersome (requiring tens of minutes to hours) and are often performed removed from the point of care. Biosensors represent a promising alternative but often fail to respond to specific targets when challenged in biological sample matrices. This failure is typically a result of biofouling, which masks the true sensor response. This talk focuses on the rational design and tuning of electrochemical, aptamer-based sensors for the detection of therapeutic levels of the antibiotic tobramycin directly in blood. The RNA aptamer-based sensors are coupled with biocompatible hydrogel membranes to enable long-term stability in this complex biological matrix.

### Keywords:
- Biomedical, Biosensors, Electrochemistry, Sensors

### Application Code:
- Bioanalytical

### Methodology Code:
- Sensors
To understand the molecular basis of brain functions, researchers would like to be able to quantitatively monitor neurochemicals in vivo. However, the chemical and physiological complexity of the central nervous system (CNS) presents challenges for the development of these analytical methods. We used the redox nature of neurochemicals at the electrode/electrolyte interface to form a basis for selectively monitoring neurochemicals. In this presentation, I would introduce the recent process in our group on in vivo monitoring ascorbate in rat brain.

1) Carbon nanotubes (CNTs) provide an electrode/electrolyte interface for the selective oxidation of ascorbate and, based on this, we have developed both in vivo voltammetry and an online electrochemical detecting system (OECS) for continuously monitoring ascorbate in CNS.

2) By using the CNT-based OECS, we compared the dynamic regional changes of extracellular ascorbate level in four different brain regions 1 h after global cerebral ischemia induced by two-vessel occlusion (2-VO). We also compared the change in the level of ascorbate in the different ischemia model (i.e., two-vessel occlusion (2-VO) and left middle cerebral artery occlusion (LMCAO) in striatum.

3) We also demonstrated the validity of the OECS for ascorbate detection as a platform for in vivo evaluation of neuroprotective efficiency of antioxidants by studying the dynamic change of hippocampal ascorbate during the acute period of cerebral ischemia and its responses to intravenous administration of antioxidants including ascorbate and glutathione.

Keywords: Biological Samples, Electrochemistry, Microelectrode
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Enzymatic bioelectrocatalysis involves the use of oxidoreductase enzymes at electrode surfaces to provide selective catalytic activity for sensing biological substrates. Enzymatic bioelectrocatalysis has been used for a variety of biosensing applications, including: glucose, cholesterol, ethanol, etc. Over the last decade, there has been great advances in both direct and mediated bioelectrocatalysis. This paper details how to engineer those electrodes for operation in bodily fluids and not just pristine buffer.

Keywords: Bioanalytical, Biosensors, Electrochemistry
Application Code: Biomedical
Methodology Code: Electrochemistry
The development of practical and effective techniques for detecting nucleic acids is critical for early diagnosis of various diseases. In recent years, there has been great interest in the design and fabrication of plasmonics-active biosensing platforms for a wide variety of medical applications using surface-enhanced Raman scattering (SERS). This presentation provides an overview of the development and applications of SERS-based nanosensors and nanoprobes developed in our laboratory to detect nucleic acid targets. Such nanodevices combining bio-recognition and nanotechnology open new horizons to a host of applications in medical diagnostics at the point of care, global health, molecular imaging, biology research, ultra-high throughput screening, and investigations of the therapeutic action of pharmaceutical agents.

Keywords: Biosensors, Nanotechnology, Nucleic Acids, Surface Enhanced Raman
Application Code: Biomedical
Methodology Code: Sensors
It is possible that one can conjugate plasmonic nanoparticles to bind to specific location in the cell. The changes in the SERS spectrum of phase synchronized cells can thus be recorded with time\(^1\) which enables us to image the molecular changes in the cell in this location during the full life of the cell or as it dies if drugs are present\(^2\). Some of the problems encountered with these techniques will be discussed.


Surface enhanced Raman spectroscopy (SERS) has been developed for a variety of bioanalytical applications requiring the rapid sensitive and highly specific identification of molecular components in human body fluids. SERS can be exploited for novel diagnostic of urinary tract infections (UTI) that does not require cell growth which is the slow step (one to three days) in current gold-standard strain specific determinations of causative bacteria. A bacterial enrichment procedure based on filtration and centrifugation, a portable Raman microscope, a reference library and associated identification software are the key components of this methodology that results in strain specific bacterial identification of bacteria in spiked human urine samples is accomplished in less than one hour by this SERS methodology. The observed bacterial SERS signatures are dominated by purine metabolic products of the bacterial cells. Robust antibiotic specific bacterial identification can be accomplished with this technique.

Tumor cells are well-known to exhibit high metabolic rates compared to normal, non-pathogenic cells. Characteristic SERS vibrational signatures due to molecules like adenine, hypoxanthine and NADH appear over the course of several hours from single cancer cells and the time dependence of these signatures are found to be different for pathogenic and nonpathogenic cells. Thus these SERS may be used for in vitro single cell cancer detection as well as fundamental studies of the effects of genetic or proteomic manipulation for cancer therapy efficacy evaluation.

SERS has also been developed for the trace detection and identification of human body fluids such as blood, semen, vaginal fluid and saliva for on-site detection and identification for forensic applications. SERS is found to be a sensitive and specific method for the evaluation and identification of human body fluids and mixtures which provide important timely evidence for crime scene investigators.

Keywords: Biological Samples, Spectroscopy, Surface Enhanced Raman, Vibrational Spectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
In this presentation we will demonstrate SERS in living cells to detect inorganic substances as well as biomolecules. We will use the concept of growing SERS substrates in living cells to illustrate our methods. Calibration and in vitro validations will also be presented. Examples of SERS in bacteria, mammalian cells, and plant cells will be demonstrated to detect a variety of events: genetic materials, proteins, inorganic substances. When appropriate, multiplex sensing will also be demonstrated.

Keywords: Agricultural, Biological Samples, Biosensors, Raman
Application Code: Nanotechnology
Methodology Code: Molecular Spectroscopy
There is a significant effort to utilize surface-enhanced Raman scattering (SERS) for medical and biomedical diagnosis and theranosis in recent years. One of the areas where has been a substantial research effort to use SERS for microorganism detection and identification. The SERS spectra acquired from whole microorganisms can be used for their label-free detection and identification. Although a capture scheme utilizing an antibody or an aptamer is necessary for their detection, it may not be need at a clinical setting and only microorganisms collected from the culture media might be satisfactory for the microorganism identification. The primary goal in clinical microorganism identification is the speed and the reliability. In this presentation, the current status and potential of the technique for clinical microorganism identification will be discussed.

**Keywords:** Biomedical, Medical, Surface Enhanced Raman, Vibrational Spectroscopy

**Application Code:** Biomedical

**Methodology Code:** Vibrational Spectroscopy
Flame retardant chemicals are often added to resins and polymers used in consumer products to reduce their flammability. Their increasing use over the past decades has led to increasing concentrations of several classes of flame retardants in both the environment and in human tissues. However, identifying consumer products that contain these chemicals, and understanding their links with human exposure, has been particularly challenging. To this end we have developed mass spectrometry-based approaches to identify flame retardant additives in polyurethane foam and textiles. Using a combination of gas chromatography low-resolution mass spectrometry and liquid chromatography high-resolution mass spectrometry, we have developed a screening method to detect the presence of 12 different organic based flame retardants that are commonly used in polyurethane and textiles. To date we have analyzed approximately 250 polyurethane foam samples and 6 textiles samples. The most common commonly detected chemicals in foam are tris (1,3-dichloro-isopropyl) phosphate (TDCPP), tris (1-chloro-isopropyl) phosphate (TCPP), a suite of polybrominated diphenyl ethers (PBDEs), and a mixture of brominated aryl esters and aromatic organophosphate chemicals associated with several different commercial mixtures. Textile materials in contrast primarily contained either tetrabromobisphenol A (TBBPA) or hexabromocyclododecane (HBCD). These chemicals are frequently identified in indoor dust and recent studies have identified these chemicals, or their metabolites, in human tissues. This presentation will provide an overview of the methods used to identify flame retardant chemicals in these samples and how their relative detection frequency corresponds to the detection and relative abundance of these compounds in human tissues.

Keywords: Environmental Analysis, GC-MS
Application Code: Environmental
Methodology Code: Mass Spectrometry
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**Abstract Text**

Numerous examples from the literature concerning environmental contaminants like antibiotics, surfactants, and pesticides suggest that biological effects can be expected within families of closely related structures, with differences in chemical structure modulating the degree and character of induced bioactivity. The possibility for conserved bioactivity within transformation products generated by either environmental or engineered mechanisms implies that characterizing the environmental significance of these products needs both chemical and biological approaches to fully understand environmental risk. To investigate the formation of bioactive transformation products within families of potent steroid hormones used as veterinary and human pharmaceuticals, we first used spectroscopic and spectrometric techniques, including high resolution, accurate mass spectrometry to initially characterize transformation products. For selected examples of major products, NMR techniques and organic synthesis was used for structural elucidation and the creation of pure standards for subsequent biological assessment. Product mixtures and isolated products were then evaluated with bioassay techniques including both in vitro and in vivo systems. These data indicate that for a number of steroid pharmaceuticals, we can expect the formation of at least some highly bioactive transformation products arising from typical environmental mechanisms. Although contaminant transformations can reasonably be expected to reduce bioactivity within a single endpoint, product bioactivity can span several different receptor endpoints and magnitudes. Thus, a comprehensive analysis of both product bioactivity and stability is sometimes needed to improve our assessment of environmental risk by specifically accounting for transformation products and non-target receptor interactions for potent pharmaceuticals.

**Keywords:** Bioanalytical, Environmental Analysis, Environmental/Water

**Application Code:** Environmental

**Methodology Code:** Mass Spectrometry
Recent studies have suggested that hydroxylated metabolites of polybrominated diphenyl ethers (OH-PBDEs) accumulate in human serum at levels similar to or greater than their parent PBDEs, and have the potential to cause endocrine disruption. Conventionally, gas chromatography/mass spectrometry (GC/MS) after derivatization is used to analyze for OH-PBDEs. However, there is a need for the direct analysis of OH-PBDEs at relatively low concentrations in environmental and biological samples. Therefore, we explored the use of supercritical fluid-based Ultra Performance Convergence Chromatography coupled to Time of Flight Mass Spectrometry (UPC\(^2\)/TOF MS) towards a non-targeted analysis to identify metabolites formed during in vitro metabolism of PBDE-100 by a recombinant human cytochrome P450 (CYP2B6). Without prior derivatization, five monohydroxylated metabolites were effectively separated by UPC\(^2\). Additionally, MS\(^{E}\), which records data without discrimination or pre-selection, was used to generate and analyze the accurate masses of fragment ions containing bromine isotopic patterns. UPC2/TOFMS proved effective in the targeted analysis of OH-PBDEs in human serum samples.
Biomolecules are abundant in the natural environment due to organism wastes and breakdown products. A number of “pollutant” biomolecules, however, can cause public health problems when present in drinking water. Genetic material that contains antibiotic resistance genes (ARGs), for example, may lead to the spread of antibiotic resistance in pathogenic bacteria. Likewise, virus particles with intact genomes can cause a range of diseases when ingested. The drinking water industry relies heavily on disinfecting treatments to destroy these biological contaminants. Interestingly, research has suggested that some disinfecting treatments (e.g., chlorine, chloramines) can actually lead to bacterial populations with increased antibiotic resistance. And although the reactivity of virus proteins with disinfectants has been reported, a thorough understanding of the fate of virus genomes during disinfection is still lacking.

Typically, virus nucleic acids and bacterial antibiotic resistance genes (ARGs) are assayed and quantified with PCR-based methods. Although these methods can be quantitative and can identify regions where nucleic acids have incurred damage, they do not identify the specific location (i.e. nucleotide) in the virus genome or ARG sequence where chemical modifications have occurred and they cannot detect the specific chemical modifications.

Here, we have employed nucleic acid mass spectrometry techniques to study the reactions that take place in virus genomes and bacterial ARGs during common disinfecting treatments. Both MALDI-TOF and Orbitrap MS analyses have been applied in order to identify regions in viral RNA and in bacterial plasmids containing ARGs that are most susceptible to chemical oxidants and UVC. We compare the degradation data collected with mass spectrometry to the degradation data collected with qPCR and to the loss of virus infectivity and ARG transformation efficiency.

Keywords: Environmental/Water, Mass Spectrometry, Nucleic Acids, Protein
Application Code: Environmental
Methodology Code: Mass Spectrometry
The rates at which different wastewater treatment plant (WWTP) microbial communities biotransform specific substrates can vary by orders of magnitude. However, it is unclear what factors lead to differences in specific biotransformation rates. We hypothesized that differences in taxonomic composition among WWTP communities may be predictive of the differences in the rates of some types of biotransformations. Here we present a novel framework for establishing predictive relationships between specific bacterial 16S rRNA sequence abundances and biotransformation rates. We selected ten WWTPs with substantial operational variation and measured the [i]in situ[/i] ammonia biotransformation rate constants in nine of them. We also selected ten micropollutants that undergo different types of biotransformations and measured biotransformation rate constants in batch reactors seeded with each of the ten WWTP microbial communities. We isolated total RNA and analyzed 16S rRNA sequence reads. We then developed multivariate models between the measured abundances of specific 16S rRNA sequence reads and the ammonia and micropollutant biotransformation rate constants. We constructed model scenarios that systematically explored the effects of model regularization, model linearity and non-linearity, and aggregation of 16S rRNA sequences into operational taxonomic units (OTUs) as a function of sequence dissimilarity threshold (SDT). For ammonia biotransformation rate constants, a large percentage of model scenarios resulted in well-performing and significant models at intermediate SDTs of 0.13-0.14 and 0.26. The 16S rRNA sequences consistently selected into the well-performing and significant models at those SDTs were classified as [i]Nitrosomonas[/i] and [i]Nitrospira[/i] groups. We then extend the framework by applying it to the biotransformation rate constants of the ten micropollutants. We identified phylogenetic groups that were robustly selected into all well-performing and significant models constructed with biotransformation rate constants of isoproturon, propachlor, ranitidine and venlafaxine. These phylogenetic groups can be used as predictive biomarkers of WWTP microbial community activity towards these specific micropollutants. This work is an important step towards developing tools to predict biotransformation rates in WWTPs based on taxonomic composition and was supported by grants from Eawag (Category: Seed) and the Korean-Swiss Science and Technology Cooperation.

Keywords: Biological Samples, Environmental/Waste/Sludge, Environmental/Water, Genomics
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
Water and wastewater disinfection processes can aid in minimizing the transport of antibiotic resistant bacteria (ARB) within environmental systems. However, the antibiotic resistance genes (ARGs) associated with such ARB may in some cases “survive” disinfection and remain capable of disseminating resistance traits to downstream bacterial communities via horizontal gene transfer processes (e.g., natural transformation). Using a combination of culture-based assays and quantitative real-time PCR (qPCR), we have confirmed that ARGs in extracellular and intracellular genomic DNA of an antibiotic resistant [i]Bacillus subtilis[/i] strain remain nearly 100% intact and capable of conferring resistance traits to non-resistant [i]B. subtilis[/i] strains via natural transformation following treatment with disinfectants exhibiting negligible direct reactivity toward DNA (e.g., NH[sub]2[/sub]Cl, ClO[sub]2[/sub]), even at exposure levels far beyond those achievable in practice. In contrast, DNA-reactive disinfectants such as HOCl, O[sub]3[/sub], and UV[sub]254nm[/sub] light are capable of degrading and eliminating the transforming activity of ARGs under practical conditions. Following exposure to each disinfectant, measurements of residual ARG transforming activity generally correlate well with qPCR measurements for amplicons of sufficient size (~800 bp) and base content to accurately capture damage capable of yielding elimination of ARG transforming activity, suggesting that qPCR analyses alone may be sufficient to confirm elimination of ARG transforming activity during the use of such disinfectants in water and wastewater treatment. While this work primarily addresses engineered water treatment applications, the general approach applied here can also be adapted to characterize the fate of ARGs during exposure to commonly-used healthcare disinfectants, as well as during natural attenuation processes (e.g., solar irradiation) relevant to environmental aquatic systems.

Keywords: Bioanalytical, Environmental/Biological Samples, Environmental/Water, Nucleic Acids
Application Code: Environmental
Methodology Code: Other
The occurrence of human and veterinary pharmaceuticals in the environment has been a subject of concern for the past two decades because many of these emerging contaminants have been shown to persist in soil and water. This concern has accelerated recently as improvements in analytical methods coupled with larger scale surveys revealed the broad range of apparently persistent pharmaceuticals that are cycling through our local wastewater-to-drinking water cycle. While recent studies indicate that pharmaceutical contaminants can pose long-term ecological risks, many of the investigations regarding risk assessment have only considered the ecotoxicity of the parent drug, with very little attention given to the potential contributions that metabolites and other transformation products may have. The scarcity of available environmental data on the transformation products of pharmaceuticals in the environment can be attributed to the difficulty in analyzing trace amounts of previously unknown compounds in complex sample matrices. However, with the advent of highly sensitive and powerful analytical instrumentation that have become available commercially, it is likely that an increased number of pharmaceutical transformation products will be identified and included in environmental risk assessment. This presentation will focus on the applications of liquid chromatography/mass spectrometry on the analysis of pharmaceutical contaminants in the influent and effluent of wastewater treatment systems. It is also intended to provide specific strategies to facilitate identification of unknown contaminants in wastewater samples.

Keywords: Agricultural, Environmental Analysis, Environmental/Waste/Sludge, Ultratrace Analysis
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
Quantum Cascade Lasers (QCL) were first realized over 20 years ago at Bell Labs, yet the technology is just now starting to find commercial applications in analyzers and instruments. As a semiconductor technology producing mid-IR light of high spectral power density and radiance, the potential for compact, high power, high-resolution devices are promising. Tiny in size at the millimeter scale, these devices are fabricated from existing semiconductor processes that promise low costs in quantity. Despite their small size, low power efficiency (~1%), thermal management challenges, and mechanically driven external cavities used for wavelength selection represent challenges to their use in handheld devices. However, the technology continues to evolve and new approaches to wavelength tuning and power efficiency should make them viable candidates for handheld devices. In this paper we will review the current state of QCL technology and explore the future of these novel devices.

Keywords: Infrared and Raman, Laser, Spectroscopy
Application Code: General Interest
Methodology Code: Portable Instruments
This paper will describe applications and field tests of handheld standoff methane sensors for enhancing coal miner safety. The sensors are based on backscatter Tunable Diode Laser Absorption Spectroscopy.

**Keywords:** Coal, Fuels\Energy\Petrochemical, Portable Instruments, Spectrometer

**Application Code:** Safety

**Methodology Code:** Portable Instruments
Handheld Spectrometers – New Techniques, New Instruments

Handheld LIBS: Development of a Fully Self-Contained, High Resolution, Gated, and Purged Instrument

The overall size of Laser Induced Breakdown Spectroscopy (LIBS) Instrumentation has been steadily shrinking over the last decade resulting in the introduction of several handheld units within the last two years. In designing any handheld instrument, trade-offs in features and functions must be made in order to accommodate the “handheld” size and form factor. We have carefully considered typical LIBS features including laser power/rep-rate, laser rastering, time-gating, spectrometer resolution/range, argon purge, data processing/OS and battery lifetime in the development of the SciAps LIBS handheld system. In this presentation, we will review those considerations along with the subsystem development paths followed to realize those requirements. Selected examples of collected data will be presented from a variety of sample types including some quantitative studies.

Abstract Text

Keywords: Atomic Emission Spectroscopy, Elemental Analysis, Plasma Emission (ICP/MIP/DCP/etc.), Portable Instruments

Application Code: Other

Methodology Code: Portable Instruments
Handheld XRF spectrometry is a mature technology for elemental chemical analysis, dating from the early 1900s. Field portable XRF is a more recent development, a little more than 40 years old. The last two decades were signified by the rapid pace of development in handheld XRF analyzers, large isotope based two-component systems of the early nineties were replaced by the lightweight; X-ray tube based one-piece handheld devices. Small silicon detectors and miniature x-ray tubes combined with the modern microprocessor and battery technologies made this evolution possible. The latest major development was the introduction of silicon drift detectors around five years ago, which improved the speed of analysis and light element performance significantly.

It is estimated that more than 50 thousand of handheld XRF analyzers are presently used throughout the world. The majority of them is used for analysis and identification of metal alloys, but handheld XRF technology has become an important tool also in many other diverse applications ranging from ore exploration to screening toys for hazardous elements. Speed of a single analysis, which translates into a large number of tests, its non-destructive character and the economics of use, are key features that have made handheld XRF analyzer a tool of choice for so many applications.

Handheld XRF is today clearly a mature analytical method, but this doesn’t mean that pace of development would have slowed down. In this presentation, we briefly review the evolution of handheld XRF and look at the science and technology of the latest generation of handheld XRF analyzers, their new applications and new analytical capabilities. In addition to ever improving analytical performance, latest technology provides smart connectivity and other new features in smaller and lighter package, making the handheld XRF technology more powerful and versatile analytical tool than ever before.

Keywords: Elemental Analysis, Instrumentation, Metals, X-ray Fluorescence
Application Code: Quality/QA/QC
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Handheld Spectrometers – New Techniques, New Instruments

Automated Colorimetric Handheld Spectrometers

Much of the miniaturization of handheld spectrometers has come from existing technology or laboratory instrumentation being shrunked to the size of an acceptable portable solution. Examples of this would be IMS, Raman, FT-IR, AFP, Chemiluminescence and Near-Infrared. Colorimetric Detection is well established as a manual technique but the application of making it Automated (without the use of the user’s eye) for detection is rather new; especially the miniaturization and reliable detection utilizing a spectrometer with applied algorithms based on expected colorimetric reactions. It could be said that it is the automating of a chemist mixing chemicals to produce a calculated result.

Colorimetric Detection has been around for many years and has undergone many iterations of development to keep up with the times and additions or variations of explosives, drugs, and other items of interest. Whereas other Handheld Spectrometers already have the semi-complete spectrum and require the library or instrumentation to be tuned to these molecules or signals, colorimetric spectrometers require a chemical reaction to be developed that creates a unique color but ignores other non-wanted items ie interferents from reacting in the visible or near visible color spectrum. Automating this is where the techniques and instrumentation become vital to the detection capability. Repeatability and chemical delivery are key and critical to making the handheld device reliable. The advantage of automated colorimetric technology is that the reaction is quite rugged and happens reliably and tolerates a variety of conditions and while it requires a finesse delivery of chemicals, the reaction is rugged and dependable allowing the Handheld automated colorimeter to be dependable and portable in many environments.

Keywords: Detector, Portable Instruments, Spectrometer, Spectroscopy
Application Code: Homeland Security/Forensics
Methodology Code: Molecular Spectroscopy
Mass spectrometry is crossing the handheld chasm. Novel micro-scale ion-trap geometries have recently been demonstrated operating at pressures 3-4 orders of magnitude higher than conventional ion trap mass spectrometers, and these pressure regimes fundamentally alter vacuum pumping burdens enabling to self-contained MS systems weighing 2-4 pounds with continuous flow rates of >3 sccm. The theory and history of these micro-scale trap advances will be reviewed, along with recent engineering and performance achievements leading to the integrated handheld systems in the field today. Tradeoffs in performance, size and automation will also be reviewed in the context of results and examples from prominent high-throughput public/private safety applications.

Keywords: Mass Spectrometry
Application Code: Safety
Methodology Code: Mass Spectrometry
Realizing small form factor and high performance in a spectrometer while seeking lower cost points has been a major challenge. New miniature spectrometer architectures are enabled by Texas Instruments DLP® technology. The ability to apply programmable filtering techniques and using high speed, high accuracy light modulation with MEMS based architectures is a unique opportunity. Several options including the latest developments in using TI DLP® products for miniature spectrometer architectures will be presented.
The continued advances of handheld instrumentation are providing significant additional capabilities for unknown chemical identification in the field.

Handheld Raman and FTIR devices have evolved since their initial release a decade or so ago, they are now globally deployed and in use by a multitude of user groups, including, but not limited to the military, customs, police, pharmaceutical and chemical industries.

The latest evolution of these devices will be discussed, with examples of the hardware, software and application advances, when compared with previous generation devices.

Keywords: FTIR, Raman, Vibrational Spectroscopy
Application Code: Homeland Security/Forensics
Methodology Code: Vibrational Spectroscopy
New drug applications (NDAs) and accelerated new drug applications (ANDAs) require the applicant to provide acceptable analytical methods to assay the product for the active pharmaceutical ingredient (API) and for related manufacturing impurities and degradants. Analytical methods need to be robust, accurate and suitable for use by the applicant and the regulatory agency. Method validation can be aided by the use of quality by design principles to demonstrate the robustness of the method. Commonly encountered reviewer observations regarding validated methods submitted to the U.S. Food and Drug Administration will be discussed.
Bioanalysis provides critical data for toxicokinetic, pharmacokinetic and pharmacodynamics studies. The demand for reliable bioanalytical methods continues to grow as a result of changing regulatory requirements and the desire to improve pharmacokinetic-pharmacodynamic predictions in humans. In addition, the challenges are ever increasing as there are an increasing number of peptide and protein drug candidates which require more bioanalytical resources. LC/MS/MS is still the most widely used technique to quantify small molecule compounds in biological matrixes and is gradually supplanting ligand binding assays for quantification of some peptide and protein drug candidates due to increased selectivity and dynamic range.

We have been working to develop processes that can be implemented to support GLP methods for small and large molecules. We have automated sample processing for both types of analytes using protein precipitation, solid phase extraction, liquid-liquid extraction and immunoaffinity purification. In addition to sample processing, we have improved post acquisition processes through implementation of electronic notebooks and new tools to automatically generate validation and bioanalytical reports. We have streamlined a drug candidate method development process to leverage knowledge from discovery methods that could then be applied toward development of robust methods for the support of preclinical (GLP) and clinical studies.

We will present an overview of the current state at our department and provide examples of challenges we faced along the way: adsorption of peptides during sample processing, accommodating different sample preparation methods into a single robotic method, and ensuring method ruggedness for small and large molecule analyses.

**Keywords:** Bioanalytical, Liquid Chromatography, Mass Spectrometry, Validation

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Febuxostat is a novel non-purine selective inhibitor of xanthine oxidase (XO), which is used for the treatment of gout. In the present study, a rapid and sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for simultaneous determination of febuxostat and its three active metabolites, hydroxylated febuxostat 67M-1 and 67M-2, carboxylic acid 67M-4 in human plasma has been developed and validated for the first time. After addition of internal standard (IS)losartan, plasma samples were pre-treated by one-step protein precipitation with methonal. Febuxostat, three metabolites and the IS were separated on a ZORBAX SB-C18 column (50mm×4.6mm, 5µm) using gradient elution with a mobile phase consisting of acetonitrile, water and formic acid. Detection was performed on a triple quadrupole tandem mass spectrometer by selective reaction monitoring (SRM) mode to monitor the precursor-to-product ion transitions of m/z 317→261 for febuxostat, m/z 333→261 for 67M-1 and 67M-2, m/z 347→261 for 67M-4 and m/z 423→207 for I.S. respectively using a positive electrospray ionization (ESI) interface. The total run time was about 12 min per sample. The method was validated over the concentration range of 10–20,000 ng/mL for febuxostat, 1.0–270 ng/mL for 67M-1 and 67M-2, and 0.8–250 ng/mL for 67M-4 respectively. The extraction recoveries of all the analytes at three concentration levels were consistent. The intra-day and inter-day precisions of the investigated components exhibited an RSD within 14.7%, and the relative errors ranged from 4.3% to 5.1%. The proposed method has been successfully applied to a clinical pharmacokinetic study of febuxostat in Chinese volunteers after oral administration of a single dose of febuxostat at 40, 80 and 120 mg.

Keywords: Bioanalytical, Liquid Chromatography/Mass Spectroscopy
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
High Throughput Drug Analysis by LC/MS: Validation and Challenges

Fast Detection and Identification of Totally Unknown Drugs, Metabolites and Other Xenobiotics Using HRMS-Based Data Mining Technology: Current Status and Challenges

Drug metabolite detection and characterization are an integral part of the drug discovery and development processes. High resolution mass spectrometry (HRMS) has emerged as the primary analytical tool for metabolite identification during the evolution of LC/MS technology in the past decade. In addition to the advances of HRMS instrumentations, newly introduced data acquisition and data mining algorithms made it happened. The metabolite identification process by HRMS usually involves three steps: (1) data acquisition to collect full MS and MS/MS spectra of metabolites, (2) data mining to find metabolite ions and retrieve their MS/MS spectra from acquired datasets, and (3) data interpretation for metabolite structure assignment. Selective detection of minor or trace amounts of metabolites in the presence of high levels of background noises and endogenous components is a challenging task. Furthermore, detection of totally unknown drugs, metabolites and other xenobiotics in complex biological systems is even more challenging. In this presentation, advantages and limitations of various data acquisition and data mining tools in analysis of totally unknown xenobiotics will be compared. Examples will be illustrated to demonstrate the effectiveness of background subtraction and metabolomic approach. Future application of these technologies to the detection of various xenobiotics, such as herbal medicines, designer drugs, abused drugs and environmental components will be discussed.

Keywords: Drugs, High Throughput Chemical Analysis, Liquid Chromatography/Mass Spectroscopy, Natural Prod
Application Code: Drug Discovery
Methodology Code: Liquid Chromatography/Mass Spectrometry
A new and aggressive focus on medicine – personalized – is emerging. Prevention and early diagnosis of disease appear to be the straightforward path toward accurate and efficient healthcare. Early detection of disease will lead to earlier treatment and followed by a response-guided medication regimen that will lead to more effective treatment. The unique opportunities for analytical technology to enable personalized medicine via early detection and response-guided medication appear to have relevance in for laboratories that require high throughput drug analysis. Recent experiences and perspectives, derived mainly from the clinical laboratory environment, will be shared in order to describe unique workflows that feature: fully integrated and automated platforms from sample handling to information out; no sample preparation; no reagent preparation; pre-validated and optimized analytical methods that require little-to-no training; and laboratories filled exclusively with FDA approved analyzers. Specific examples will be presented to demonstrate how analytical platforms such as a mass spectrometer can be transformed into a clinical analyzer – the molecular microscope and molecular plate reader – to enable unprecedented levels of high throughput drug analysis, and perhaps, the future of personalized medicine.
A clear understanding of the absorption and metabolism of a traditional Chinese medicine (TCM) is very important in its clinical use and pharmacological research. To find more of the absorbed constituents and metabolites of a TCM, a novel strategy was proposed. This strategy consisted of three steps, including (1) the construction of a digital library of PRR, which included the parent compound database, the known metabolites database, the characteristic neutral losses database, (2) searching the absorbed constituents and metabolites by comparing the extracted ion chromatograms (EICs) and base peak chromatograms (BPCs) of the test group with those of the control group and collected the related information, and (3) the identification of absorbed constituents and metabolites. The absorbed constituents and known metabolites were identified with the digital library described above. The unknown metabolites were elucidated in several steps: first, the conjugation type was identified according to the characteristic neutral losses in MS; second, the skeleton structure was elucidated by querying the CAS Registry and other bioinformatic tools using the molecular formula; third, the proposed structure was further confirmed by MSn fragment ions and maximum UV absorption; and finally, the exact conjugation site was determined for some metabolites based on the ClogP and predominant conjugation site.

This strategy was first applied to screen and identify the absorbed constituents and metabolites of a commonly used TCM Paeoniae Radix Rubra (PRR) decoction and paeoniflorin in rats. In total, 13 new absorbed constituents and 90 new metabolites of PRR decoction were detected. Among these metabolites, the structures of 70 metabolites were identified, and the conjugation types and structure skeletons of the other 20 metabolites were preliminarily determined. These findings enhance our understanding of the metabolism and effective forms of PRR decoction and paeoniflorin.

Keywords: Database, Liquid Chromatography/Mass Spectroscopy, Natural Products, Plasma
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Hepcidin is a 25-amino acid peptide hormone with 4 cysteine bridges. It is produced in the liver to regulate iron homeostasis. Hepcidin is excreted to urine during chronic renal inflammation because of incomplete or impaired reabsorption. The level of urinary hepcidin-25 has been identified as a potential biomarker for kidney damage in previous reports. Because of the structural similarity between hepcidin and its metabolites (hepcidin-20, 22 and 24), an LC-MS assay would easily provide better specificity than a ligand binding assay. In this work, a sensitive and robust LC-MS/MS assay has been developed and validated to measure hepcidin in human urine over the concentration range of 0.5 to 500 ng/mL. Special procedures have been used to overcome challenges such as matrix effect, absorption and carryover that were encountered during the assay development and validation. The assay has been validated to support clinical research.
It is well-known that transporters play a vital role in the ADMET profile of many drugs. Because of this, it is necessary to develop and implement panels of relatively high-throughput (HT) transporter inhibition assays to screen for potential liabilities earlier in drug discovery in an effort to inform lead chemotype selection. Using a 384-well format, these inhibition assays can be conducted in freshly-plated cryopreserved cell lines and/or vesicular systems expressing the transporter of interest. Currently, many of these assays use microplate-based scintillation counting to assess probe substrate transport, but in order to reduce the cost and waste streams associated with using radiolabelled drug substrates, a MS-based solution is necessary. Analysis with traditional LC-MS/MS allows the use of clinically-relevant probe substrates to assess compound inhibition of transporter activity, but typically does not offer the throughput needed to support the high sample volumes associated with an early liability screen.

One solution for improving throughput is to couple SPE with MS detection using a RapidFire™ (RF) system. By using ultrafast RF analysis, it is possible to easily support multiple transporter inhibition assays in early drug discovery. Hepatobiliary transporter inhibition assays have been developed for OATP1B1 and NTCP using the RapidFire platform. RF methods are inexpensive when compared to radiolabeled assays and are much faster than traditional LC-MS/MS methods, yet demonstrate similar sensitivity, selectivity, reproducibility, linearity and robustness. Validation with reference compounds demonstrates that these assays are robust, reproducible, and generate data consistent with literature, LC-MS/MS, and radiometric values. Implementing this type of HT-assay panel may facilitate informed clinical candidate selection and could establish a foundation upon which one can build a better understanding of the role that transporters play in ADMET biology.

Keywords: Bioanalytical, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Differential mobility spectrometers provide characterization and separation of gas ions derived from sample vapors based on field dependent mobility coefficients between 10 to 150 Townsend at ambient pressure in air. In a tandem configuration, DMS analyzers can be used to transmit ions selectively through the analyzer according to field dependent behaviors described in dispersion plots, or plots of ion current, compensation field, and separation field. Since ions exhibit field dependent mobility with distinctive dispersion plots, one combination of separation and compensation fields will filter or remove all ions in a DMS stage except those with a favorable match in differential mobility to the two fields. In purified air at 1 to 100 ppm moisture, field dependences can be significant between 50 to 150 Td and two DMS stages can remove all ions except those with favorable matches to two combinations of separation and compensation fields. Since filtering occurs based on non-linear dependences of mobility with separation fields, the tandem DMS detector is more than a sequential filter and holds implicit selectivity rooted in hydration of ions. Response in the tandem DMS begins with chemical ionization processes which are simplified by chromatographic pre-separation and the tandem DMS provides a measure of selectivity not attainable with single stage mobility detectors and with theoretical response times of ~10 ms. The addition of either ion fragmentation or vapor modifiers between the first and second DMS stages can provide a further level of selectivity for targeted substances.

Keywords: Capillary LC, GC Detectors, Instrumentation, Volatile Organic Compounds
Application Code: General Interest
Methodology Code: Other
Ion mobility techniques (IMS, AIMS and DMS) share simplicity, high speed, and a broad range of scientific and analytical capabilities for detection of trace individual chemicals in mixtures. Nevertheless experience shows that analytical power of standalone mobility based instrument’s is insufficient to satisfy current, enhanced requirements when they used in harsh environment conditions, e.g., airport, shopping malls and subway systems. In such environments, the false alarm rates of the ion mobility analyzers become too high, due to the presence of numerous interferents in the raw, real-world samples. One of effective ways to enhance the performance of analysis is to add orthogonal chemical characterization by combining the other well developed analytical methods to ion mobility measurements. Integrating two orthogonal physical–chemical views of analyte and interferents usually enhances the analytical performance of the combined system. According to its principle of operation, the DMS sensor can operate in two regimes: a) as a spectral detector and/or b) it can be used as a tunable non-distractive ion pre-filter for selection of specific ion species. This provides flexibility to build different hyphenated systems on the basis of DMS with many analytical systems.

In this talk, we will overview and present descriptions and analytical characterization of existing hybrid systems which were developed, tested, and produced on the basis of a planar DMS design sensor. These are the following hyphenated systems: a) GC-DMS, b) DMS-AIMS, DMS-DMS, DMS-IMS2, and DMS-MS. The current prototypes have high sensitivity (ppb level) and enhanced robustness for operation in field conditions for a wide range of chemicals including CWA simulants, explosives, TICs and TIMs, and environmental pollutants.

Keywords: Analysis, Instrumentation, Portable Instruments, Sensors
Application Code: Homeland Security/Forensics
Methodology Code: Other
Environmental monitoring for manned spaceflight has long depended on archival sampling, which was sufficient for short missions. However, the longer mission durations aboard the International Space Station (ISS) have shown that enhanced, real-time monitoring capabilities are necessary in order to protect both the crewmembers and the spacecraft systems.

Over the past several years, a number of real-time environmental monitors have been deployed on the ISS. Currently, volatile organic compounds (VOCs) in the station air are monitored by the Air Quality Monitor (AQM), a small, lightweight gas chromatograph-differential mobility spectrometer. For water monitoring, real-time monitors are used for total organic carbon (TOC) and biocide analysis. Presently, no information on the actual contaminants contributing to the TOC value is provided.

An improvement to the current state of environmental monitoring could be realized by modifying a single instrument to analyze both air and water. As the AQM currently provides quantitative, compound-specific information for VOCs in air samples, this instrument provides a logical starting point to evaluate the feasibility of this approach. The major hurdle for this effort lies in the liberation of the target analytes from the water matrix.

In this presentation, we will discuss our recent studies, in which an electro-thermal vaporization unit has been interfaced with the AQM to analyze target VOCs at the concentrations at which they are routinely detected in archival water samples from the ISS. We will compare the results of these studies with those obtained from the instrumentation routinely used to analyze archival water samples.

Keywords: Air, Environmental, Water

Application Code: Environmental

Methodology Code: Other
### Abstract Text

Introduction: Under ideal circumstances the species that arrive at the detector during an ion mobility experiment represent the starting conditions. However, the presence of metastable species and solvating neutrals are experimental realities that result in deviations from the ideal. Despite these challenges, ion mobility-mass spectrometry (IMMS) systems represent a powerful tool to probe gas-phase equilibria and in some cases the thermochemistry of systems. This work details the experimental subtleties of implementing the Fourier transform IMMS experiment which eliminates the observation of reaction intermediates and simplifies spectral interpretation. Specifically, we demonstrate the utility of this approach to characterizing gas-phase ion clustering behavior with the standard DMMP and an unknown system using tributyl-phospate (TBP).

**Methods:** Fourier experiments were conducted using an atmospheric pressure IMMS system operated at WSU. The drift tube of this system operates at a range of temperatures from 100-250°C with electric fields below 3Td. Following ionization using electrospray and a desolvation period, DMMP and TBP and ions were gated into the 23 cm-long drift tube connected to a TOF mass spectrometer. Following an experimental design analogous to Tabrizchi the drift gas was modified with neutrals using a custom drift gas inlet. Signal averaging experiments used gate pulse widths of 210\(\mu\)s and analogous FT spectra were obtain by sweeping the ion gate frequency from 10–10,000 Hz over the period of 1s.

**Preliminary Data:** Serving as a thermometer ion the dimer of DMMP was observed in the IMS-TOF when the drift gas was modified with the neutral analyte. During the standard signal averaging mode a pronounced tail is traced between the monomer and dimer, however, under the FT-IMMS mode of operation these reaction intermediates are removed. Observation of the TBP dimer has been achieved and a detailed characterization of this clustering equilibria underway.

**Keywords:** Instrumentation, Mass Spectrometry, Other Hyphenated Techniques, Time of Flight MS

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Other
Abstract Text

Ion Mobility Spectrometry is the leading technology for trace detection to prevent unauthorized transport and use of dangerous materials to include explosives, toxic chemicals, illegal drugs and other forms of contraband. The primary complaint by users of IMS detectors, or any trace detection technology, is false alarms. By measuring the mobility to an improved accuracy of an order of magnitude, effective resolution of ion mobility spectrometers can be improved while maintaining high sensitivity without expensive hardware modifications.

Accurate and precise reduced ion mobility constants, K0, are being obtained for target compounds with accurate and precise measurements of temperature, pressure, electric field strength (voltage and ion drift distance), and drift gas water concentration in positive and negative ionization modes – precision and accuracy of measurements is an order of magnitude better than literature values. Parametric measurements are made using commercial NIST-traceable sensors. Ion mobility constants are determined using a new, precisely constructed, ion mobility spectrometer interfaced to a time-of-flight mass spectrometer. Accurate, precise ion mobility constants result in greater spectrum peak capacity and narrowing detection spectrum “windows” to reduce potential for false alarms – detection sensitivity is not affected. Narrow windows can be incorporated in fielded detection instruments using only software and firmware modifications, significantly less expensive than hardware modifications required to achieve similar results.

Details of the construction of ion mobility spectrometer, precision and accuracy of parametric measurements, and experimental methods will be described. Target compounds include TNT, 2,4-DNT, NG, EGDN, DMNB; chemical warfare agent simulants methyl salicylate (MES), dimethyl methylphosphonate (DMMP), thiodiglycol (TDG); proposed IMS standard analyte di-tert-butyl pyridine (DtBP).

Keywords: Calibration, Detector, Monitoring, Other Hyphenated Techniques
Application Code: Safety
Methodology Code: Other
## Session Title
Hyphenated Ion Mobility Spectrometry

## Abstract Title
**High Performance Ion Mobility Spectrometry Brings a New Dimension to HPLC Separation**

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### Abstract Text
The growing interest in high resolution multi-dimensional separation is driving the development of advanced instrumental platforms that can directly handle complex samples. With the recent development of ambient-pressure high-performance ion mobility spectrometry (HPIMS), ion mobility separation alone offers meaningful analytical performance comparable to chromatography. In many on-site analytical needs, standalone IMS systems are used as an alternative to HPLC. When combined with HPLC as a detector, HPIMS can identify UV transparent compounds and offer a complimentary separation capability for coeluting compounds. The combination of HPLC-HPIMS delivers two dimensional separation based on orthogonal separating principles, providing a simple, low cost, compact analytical system to common analytical laboratories.

On the other hand, when HPIMS is coupled with mass spectrometry (MS), ion mobility spectrometry adds tremendous value to the analysis of species that were either previously may lead to undesirable, highly complex spectra. As ion movement within the IMS drift tube is governed by its size/cross-sectional area, effective separation of molecules with slight structural differences such as conformers, isobars, and isomers can be achieved. In order to maximize the advantages of this technology, we have developed, evaluated, and introduced an ambient pressure IMS device that readily integrates with the ion trap MS line of Thermo Scientific instrumentation (i.e. LTQ Velos, Orbitrap).

### Keywords:
Amino Acids, Food Safety, HPLC Detection, Pharmaceutical

### Application Code:
Food Safety

### Methodology Code:
Separation Sciences
Hyphenated Ion Mobility Spectrometry

A New Approach for the Detection and Identification of Explosives by a Combination of IMS and Raman Spectroscopy

Ion Mobility Spectrometers (IMS) are often used as trace detectors for explosives and can be found at many airports. The different time of flight of ions is used in order to differentiate between all kinds of explosives. Some military used explosives have nearly no vapor pressure and require special sampling methods. Therefore a thermal desorption unit is needed in order to vaporize explosive residues which have to be sampled by wipe pads in advance. In addition, higher operating temperatures in the detector are required. Many explosives are based on nitrogen-oxide compounds, which can be detected in the negative mode of the IMS. New peroxide based explosives, now often used in terrorist attacks due to their simple manufacture, have a much higher vapor pressure and have to be detected in the positive mode of the IMS. A new double tube IMS was developed in order to detect positive and negative ions simultaneously. IMS systems at airports are used without any pre-separation. A very fast analysis is possible, but the identification of the explosives is sometimes difficult and even more problematic are false alarms. This is why different detection technologies are often combined in order to gain selectivity. Another fast and selective technology is Raman spectroscopy. Especially the surface enhanced Raman spectroscopy (SERS) is a suitable detection technology because of the low detection limits, similar to IMS. In Raman spectroscopy the identification of explosives is based on their molecular vibrations, which is an orthogonal technique to the IMS. Therefore, a novel approach based on a combination of IMS and SERS in one instrument for the simultaneous detection of all kind of explosives will be presented. The technology and first results will be discussed.

Keywords: Detection, Surface Enhanced Raman, Trace Analysis
Application Code: Safety
Methodology Code: Other
## Hyphenated Ion Mobility Spectrometry

### Ion Modification – A New Technology to Enhance the Selectivity of TOF-IMS Detectors

Traditional time of flight Ion Mobility Spectrometers operate by making a measurement of the velocity with which ions generated from a sample travel through a linear electric field, typically 250 V/cm. The velocity with which an ion travels from an ion gate to a collector (a distance referred to as the “drift region”) is usually reported as the “reduced mobility”. The mobility of an ion is determined by its mass, its charge and its collisional cross sectional area. As such, ions generated from different compounds can be differentiated from one another based on the reduced mobility measurement.

Problems can arise when different compounds give rise to ions with very similar or identical mobilities, and the spectrometer is unable to distinguish between them. In order to eliminate the prospect of a false negative the spectrometer is usually programmed to alarm if any ion which matches the target compound mobility is detected. This subsequently can lead to false positive alarms.

Ion Modification is a new technology which can be used to substantially reduce the likelihood of false positive alarms. An IMS which utilises Ion Modification technology incorporates additional electrodes within the drift region, upon which a high amplitude high frequency waveform can be applied. The waveform subjects the ions to an electric field vastly higher than the normal IMS field. Under these conditions, the energy of the ion/gas collisions are sufficiently high for chemical rearrangements to take place, such that new ions are formed. The new ions typically have a higher mobility than the parent ion.

In the majority of cases, a modified ion from a target compound has a different mobility to a modified ion from an interferent. As such, activation of the ion modifier can lead to differentiation between compounds that otherwise could not be differentiated in a traditional IMS.

The technique has been shown to have utility in improving the detection of many classes of compounds.

**Keywords:** Chemical, Gas, Identification, Sensors

**Application Code:** Other

**Methodology Code:** Sensors
The ability to obtain accurate and reliable data is an essential aspect of scientific research. Recent trends have sought to minimize cumbersome and expensive instrumentation in efforts to free-up crucial laboratory space and finances. The Qubit[registered] quantitation platform is an inexpensive, versatile, and straightforward alternative to a benchtop fluorometer. Currently, the Qubit[registered] Fluorometer is used in conjunction with kits for the quantification of DNA, RNA, microRNA, and proteins. These quantitation kits provide unparalleled accuracy, sensitivity and selectivity for the target analyte in as little as 20 minutes. The Qubit[registered] programs utilize an algorithm that, after a quick 2-3 point user calibration, directly reports data in terms of concentration. To expand the breadth of the Qubit platform, we have recently established application notes for cholesterol and glucose detection. The programs for these detection assays can be easily uploaded to the Qubit[registered] instrument using a USB drive. Both assays combine the resiliency of Amplex[registered] Assay kits offered by Life Technologies with the ease of use offered by the Qubit[registered] Fluorometer. Using the MyQubit software and web tool, we have established several additional Qubit[registered] assays that utilize existing Amplex[registered] Assay kits, as well as other popular detection assays. Here, we will highlight the user-friendly nature of the “create-your-own-app” format and outline how any user can convert their detection method to an intuitive Qubit[registered] assay. Overall, we will demonstrate how the Qubit[registered] platform is a cost-saving replacement for a traditional benchtop instrument that has the versatility to work with any fluorescence detection scheme.

Keywords: Analysis, Bioanalytical, Biological Samples, Fluorescence

Application Code: Bioanalytical

Methodology Code: Fluorescence/Luminescence
The general benefits of working at higher than ambient temperature, including shorter analysis time, higher efficiency and lower column and system pressures, equally apply to the separation of low- and high-molecular mass compounds. In this presentation we will examine the impact of working at elevated temperature on the retention of globular proteins, monoclonal antibodies and peptides during the lifecycle of columns packed with 3.4 micron, 400 angstrom pore size, C4 alkyl-bonded, superficially-porous particles. Short 2.1mm ID columns were subjected to aqueous acetonitrile/trifluoroacetic acid mobile phase gradients at temperatures up to 90 °C for 15-60,000 column volumes, during which repeated injections of protein and peptide standards were made. Our findings suggest that at 75-90 °C the columns last longer for the analysis of globular proteins and monoclonal antibodies than for peptides in terms of the stability of retention time, while efficiency and peak shape were not or only minimally impacted. While a modest decline in retention time was observed for proteins, peptides showed a more pronounced loss of retention during long term gradient operation, a result that is expected by differences in their gradient steepness parameters. At 35 °C we did not observe a decline in retention time, nor were peak shape and efficiency impacted for any of the biomolecules studied. The results of our studies illustrate the importance of optimizing temperature when performing biomolecule separations and will prove beneficial to analysts who are tasked with finding optimum conditions for the separation of proteins or peptides during drug development and biomarker research.
Kaiser Raman Spectroscopy enables in situ measurements. In combination with Umetrics tools for multivariate modelling and visualization, real-time monitoring and control of bioprocesses is facilitated.

Data collected at a biopharmaceutical company will be presented illustrating the use of Raman spectroscopy coupled with multivariate data analysis to quantitatively monitor multiple bioprocess parameters.

New joint efforts in software tools from Umetrics and Kaiser provides a smooth and seamless data handling and integration which allows the user to easily generate, develop and implement Raman based solution for process monitoring based on chemometrics models.

**Keywords:** Bioinformatics, Chemometrics, Process Analytical Chemistry, Raman

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
Monoclonal antibodies (mAbs) are the most prominent class for protein therapeutics because of their high specificity, excellent biocompatibility and effectiveness against autoimmune disorders, cardiovascular diseases, infectious diseases, and cancer. The proliferation of monoclonal antibody therapeutics and their susceptibility to various biochemical modifications has highlighted the importance of characterizing these highly heterogeneous products for their safety and efficacy.

Hydrophobic interaction chromatography (HIC) is a technique for separation of proteins including monoclonal antibodies, and has been widely used as an orthogonal method to size exclusion chromatography and ion exchange chromatography for analysis of mAb and related substances, such as succinimides, fragments, oxidants, C-terminal lysine modification, and drug conjugates, to monitor the stability and potency of the drug.

Here we introduce a new family of HIC columns designed for mAb analysis. They exhibit desired and complementary selectivity, excellent recovery, high efficiency, and chemical stability. The effectiveness for mAb separation has been demonstrated by examples including antibody-drug conjugates (ADCs), antibody fragments, oxidized mAb, PEGlyated mAb, antibody aggregates and fusion protein.

Keywords: Bioanalytical, Biopharmaceutical, HPLC
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
In this study, a relatively simple workflow for confident identification of compounds was developed and tested on a variety of cannabis samples. Analyses included a combination of complementary hard and soft ionization methods coupled to high performance time of flight mass spectrometry, as well as, effective data processing for characterization of samples. In general, GC-TOFMS profiling of materials was an effective way to determine the relative concentration of active components, as well as, characterize anthropogenic materials in cannabis samples. Instrumental analyses were fast, reproducible and provide excellent chromatographic resolution. High resolution time-of-flight mass spectrometry (HRT) resulted in additional benefits such as robust formula determinations for fragment, quasi-molecular and adduct ions, as well as, increased selectivity that resulted in reduction of background interferences. HRT data acquired over a large mass range was probed multiple times via targeted or untargeted processing methods. Data were searched against well-established nominal mass libraries (e.g., NIST 14, Wiley 10) to facilitate confident identification of components. These results were corroborated using formulae generated from the high resolution, accurate mass data (< 1 ppm). Where possible, standards were analyzed for unequivocal confirmation of chemical formulas and their proposed structures.
Exposure of DNA to oxidative stress is responsible for the formation of 8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxodG). 8-oxodG is genotoxic and can cause cancer. It is considered to be the biomarker of oxidative stress. In this current research work a new immobilization approach has been developed for the fabrication of ECL sensors for oxidative DNA damage. Composite films containing Os(bpy)$_2$phen-COOH-Nafion-Graphene (OsNG) were assembled by drop casting on the pyrolytic graphite to develop sensors that selectively detect 8-oxodG in intact ds-DNA. The stability and reproducibility of the composite films were investigated. The electrochemistry and electrochemiluminescence (ECL) of composite films containing OsNG were also studied using sodium oxalate as a co-reactant on a pyrolytic graphite electrode. A hydrophobic cation like Os(bpy)$_2$phen$^{2+}$ could be easily incorporated into the hydrophobic nafion films, however the uptake rate of Os(bpy)$_2$phen$^{2+}$ was observed to be relatively slow. Immobilization of graphene on the electrode resulted in the high peak current and ECL intensity of Os(bpy)$_2$phen$^{2+}$, which could be due to the greater accessibility of the ion exchange sites in the composite film, hence leading to a faster rate of diffusion of Os(bpy)$_2$phen$^{2+}$ in the films. Sensor arrays were fabricated on the graphite electrode using a print and peel method. OsNG composite and the oxidized DNA films were assembled on these arrays and were finally housed in a microfluidic device, which was used to detect DNA oxidation caused by Fenton's Reagent. This simple approach could further be used to detect Copper-mediated oxidative DNA damage caused by metabolites of pollutants and drugs.

**Keywords:** Chemically Modified Electrodes, Electrochemistry, Electrodes, Lab-on-a-Chip/Microfluidics  
**Application Code:** Clinical/Toxicology  
**Methodology Code:** Electrochemistry
Clinical Chemistry Applications by Novel Techniques

Nonaqueous Microchip Electrophoresis with Online Mass Spectrometric Detection of Lipid Species

While liquid chromatography is the most common liquid separation technique for hydrophobic analytes, nonaqueous capillary electrophoresis (CE) has advantages for some analyte classes including better separation quality, quicker separation times, and more economical use of samples and solutions. Nonaqueous CE can be coupled with mass spectrometry, and moving the separation to a microfluidic chip can further enhance the speed and quality of separations. In this study, a fully nonaqueous separation buffer system is used for high quality separations of phospholipids. This separation buffer is then used on an electrospray ionization microchip electrophoresis device for coupling the liquid separation to mass spectrometry. Initial work focused on developing a proper buffer system for nonaqueous CE of phospholipid standards. Surprisingly, N-methylformamide (NMF) shows substantial solvation ability and electroosmotic flow, even in the absence of supporting electrolyte. Electrophoretic separations on a microchip were performed with fluorescently labeled phospholipids yielding a baseline separation of a quaternary lipid mixture with a full separation in less than two minutes. Coupling of the microchip electrophoresis separation with electrospray ionization mass spectrometry is accomplished using a second make-up flow channel prior to electrospray at a monolithically integrated electrospray tip. Currently, the coupling of the separation and ESI is being optimized for high quality separation with mass spectrometric, label-free detection of lipid species from NMF. Further work will target biological fluids to detect and quantify known lipid disease biomarkers and discover new potential disease biomarkers.

Keywords: Capillary Electrophoresis, Electrospray, Lab-on-a-Chip/Microfluidics, Lipids
Application Code: Clinical/Toxicology
Methodology Code: Capillary Electrophoresis
Clinical Chemistry Applications by Novel Techniques

Continuous Monitoring of Volatile Organic Compounds (VOCs) in the Breath of Mechanically Ventilated Patients by Means of Proton Transfer Reaction Time-of-Flight Mass Spectrometry (PTR-ToF-MS)

In critically ill patients, life-threatening changes of vital conditions may occur within seconds. Some of these changes translate into breath VOC profiles. Non-invasive real time monitoring might become possible when breath resolved determination of VOCs could be realized through direct mass spectrometry.

Breath from 12 mechanically ventilated patients was analyzed continuously by means of PTR-ToF-MS during one hour immediately following cardiac surgery. For safety reasons, a 6m long heated silicosteel transfer line was used for breath sampling.

Through optimization of operational parameters a time resolution of 200ms could be realized for PTR-TOF measurements. Thus, breath resolved detection of more than 300 mass traces was possible without interference with mechanical ventilation and without need of additional equipment. Identification of alveolar and inspiratory phases was performed by a custom-made algorithm. Detection limits (for continuous monitoring) were in the low ppbV / high pptV range. Exogenous contaminants (anesthetics, disinfectants) could be identified by comparison of breath phases. Analyzing specific markers, representing aspects of metabolism (acetone), cardiac output dependency (isoprene) or oxidative stress (aldehydes) we were able to monitor (patho)physiological processes non-invasively. During a lung recruitment maneuver isoprene concentrations decreased by 52% whereas PCO2 only decreased by 13%, acetone decreased by 26%. After lung recruitment acetone had decreased by 14 %, whereas isoprene and CO2 increased by 4% and 9%.

Continuous VOC monitoring by PTR-TOF enables immediate recognition of physiological or therapeutic effects in ICU patients. In a perspective non-invasive real-time monitoring of breath biomarkers may act as tool to observe patients’ clinical conditions.

Keywords: Gas, Mass Spectrometry, Medical, Time of Flight MS
Application Code: Clinical/Toxicology
Methodology Code: Mass Spectrometry
Ion-selective electrodes (ISEs) and reference electrodes (REs) are used to determine the concentrations of target ions in sample solutions. Recently, paper-based potentiometric sensors with conventional ISEs and REs have been developed to detect the concentrations of various ions (K+, Na+, Ca2+, and Cl-). In this work, we simplify the design by using all-solid-state ISEs and REs to replace conventional electrodes and fabricate a paper-based all-solid-state potentiometric ion-sensing platform. This ion-sensing platform contains an ion-selective membrane and a reference membrane, both doped with a well-defined redox couple and contacting with colloid-imprinted mesoporous carbon as solid contact. With these devices, no external reference solutions are needed and sample volumes as small as one droplet can be achieved, which greatly simplifies the operating procedures and minimizes human errors. Furthermore, these miniaturized sensors are portable, disposable and inexpensive, making them promising commercialized ion sensors for clinical analysis.
Detection and quantification of biological material is integral to biomedicine, clinical diagnostics and forensic science. We have previously developed a label-free DNA detection modality through bead aggregation, which has been firmly established at previous conferences[1,2] and in two recent seminal papers[3,4]. The dual-force aggregation (DFA) system allows for the multiplexed, rapid detection and quantitation of non-specific DNA via chiroptope-driven aggregation (CDA). The purpose of this research is to adapt and demonstrate the capability of the DFA system in clinical (cell counting) and forensic (buccal swabs and rare samples) applications.

Here, we show that the DFA system and CDA provide a simple, rapid, and accurate method for white blood cell (WBC) counting. The number of cells in a sample can be obtained by back calculating from the DNA concentration that is determined from the degree of bead aggregation. To date, in a comparison with traditional WBC counting methods, the DFA system provided accurate WBC counts within an average of 430 cells/µL and a median of 197 cells/µL of clinical values in less time, with only 10 µL of whole blood.

In addition to clinical applications, we have applied the system to buccal swab and rare blood sample analysis for forensic applications. With a newly adapted extraction procedure, DFA has shown effectiveness in quickly, and accurately, quantifying buccal samples prior to STR analysis. Further, the newly improved sensitivity of the DFA method[4] shows promise quantifying and extracting rare blood samples from fabric and other materials.

A fully integrated, small surface plasmon resonance (SPR) sensor has been developed for the therapeutic drug monitoring (TDM) of methotrexate (MTX) levels in actual clinical samples. The SPR instrument is based on the wavelength interrogation configuration with sequential lighting of the measurement channels. We have implemented a novel method with this instrument which provides direct readout of MTX concentrations in patient’s serum on a SPR sensor. The developed method consists of employing folic acid functionalized gold nanoparticles (Fa-AuNP) in competition with MTX for a specific number of its targeted enzyme, human dihydrofolate reductase (hDHFR) immobilized on the sensor surface. The sensor was calibrated with spiked MTX samples in a pool of serum from healthy patient, providing a model to mimic real clinical samples analysis. Nonspecific adsorption from serum components has been reduced by an anti-fouling peptide monolayer. Preliminary data with actual clinical samples from a local hospital shows good agreement between the SPR method and LC-MS/MS and Fluorescence Polarization Immunoassay (FPIA). Ultimately, our work will focus on integrating our SPR based sensing platform for rapid and direct analysis of sera MTX levels in patients under chemotherapy in hospital laboratories. We also demonstrate that the SPR instrument is suited for monitoring ligand-receptor interaction in drug design, to identify pheromones and to quantify hormones.
Atmospheric Methane is one of the key greenhouse gases for global warming. The urban methane concentrations are influenced by the anthropogenic emission of methane that originates from livestock, landfills, rice paddy fields, energy production and sewage treatment.

The challenge in accurate monitoring of the atmospheric methane using infrared absorption based techniques are the low detection limit requirement and selectivity in the presence of varying concentrations of water. In the proposed solution tunable quantum cascade laser source operating at the mid-infrared range is combined with cantilever enhanced photoacoustic detection scheme [1] [2] [3] . This achieves ppb-level detection limit and high selectivity due to the possibility of performing measurement at lowered pressure.

The conclusion of the suitability of the tunable quantum cascade laser combined with cantilever enhanced photoacoustics in monitoring the atmospheric concentrations of methane in the presence of interfering gas components and changing ambient conditions will be presented based on the measurement results and user experience.


Keywords: Environmental/Air, Molecular Spectroscopy, Photoacoustic, Spectroscopy
Application Code: Environmental
Methodology Code: Molecular Spectroscopy
Carbon Capture and Storage (CCS) technologies aim to reduce the release of greenhouse gases (especially CO2) generated from fossil fuel combustion. Air Liquide is a key contributor to the evolution of such CCS technologies in the field of oxycombustion, actively participating in collaborative projects for the development and improvement of CO2 Compression and Purification Units (CPU). Reliable analysis of the impurities through the whole process plays a key role to demonstrate the capabilities of oxycombustion. A mobile laboratory featuring analyzers for all major flue gas components and key impurities has been designed and installed at the Callide Oxyfuel demonstration site and connected to more than twenty different sampling points on the CPU. Different types of analyzers such as multi-component analyzers based on NDIR, FTIR and chemiluminescence were used to determine the effluent compositions at different steps in the process.

The CPU installed at the Callide power station has been operated since Q4 2012 (currently more than 3500 hours of operations) and has been optimized in order to improve plant reliability. Data were collected during two analytical campaigns (2013 and 2014) to evaluate the CPU performances. The results have been used in data reconciliation models and helped to better understand the process to further improve subsequent demonstration designs.

This presentation will illustrate the complexity of impurity measurements in CO2 rich streams with results from the test campaigns carried out in 2013 and 2014 at the Callide Coal-fired power station (Queensland, Australia).

Keywords: Energy, Process Analytical Chemistry, Process Monitoring
Application Code: Environmental
Methodology Code: Process Analytical Techniques
Aquasphere is threatened by both organic and inorganic pollutants. Adsorption of pollutants on activated carbon is one of the various successful remediation processes. Activated carbons produced from various raw materials are usually selective for any one type of pollutants. This study focuses on activation processes that can prepare activated carbon from a single substrate which is selective for both types of pollutants. Three types of activated carbons were produced from the same substrate and compared with the commercial activated carbon (CAC) for adsorption properties. AC – 01 was prepared by treating raw material with sulfuric acid at 250°C, treatment of AC – 01 with aqueous NaOH yield AC - 02. Heating AC – 01 at 850°C in an inert environment produced AC - 03. Lead(Pb), cadmium(Cd), methyl blue(MB) and xylene orange(XO) were selected pollutants for this study. It was observed that activated carbon produced at high temperature was selective for organic pollutants whereas low temperature activation yielded carbon selective for inorganic pollutants. Trend of percent removal for CAC was XO (99%) > MB (90%) > Cd (20%) > Pb (11%). The trend for AC – 03 was XO (18%) > MB (35%) > Pb (13%) > Cd (4%). Adsorption trend was reversed for AC – 02 that is XO (2%) < MB (13%) < Pb (93%) < Cd (93%). For AC – 01 the respective percent removals were XO (0%) < MB (5%) < Cd (38%) < Pb (79%). A blend of produced activated carbons can be effective for removal of both types of pollutants.
Historically, infrared (IR) spectroscopy methods were widely used for quantifying oil, grease and hydrocarbons in water samples. Infrared spectroscopy allows for fast and easy measurement of these types of samples. Various IR methods were used globally according to local regulations or protocols. Prior to the Montreal Protocol in 1989 prohibiting the use of chlorinated solvents, most of the IR methods involved the extraction of hydrocarbons in water samples used carbon tetrachloride, CCl₄, as the extraction solvent. Alternative methods were required to replace these methods, with many laboratories moving away from IR methods. Some of these methods, such as EPA 1664, still use hazardous solvents for the extraction of hydrocarbons from water samples, prior to gravimetric measurement.

A solvent-free IR method, ASTM D7575, is presented that uses an IR-transmitting membrane material that will extract and retain the hydrocarbon species as a known quantity of water sample is passed through it. The membrane is contained with a filter extraction device, called the Extractor, which has a standard Luer lock fitting for processing water samples through the membrane. After the sample is processed, the membrane is then dried by using compressed dry air. Once dry, the Extractor device is placed directly in the IR beam of the spectrometer and the hydrocarbon content determined based on a set of hydrocarbon calibration standards. The paper describes the development and implementation of a workflow to simplify the analysis process, will show examples of measurements, and also highlight some of the significant advantages of this method for this type of analysis.

Keywords: Environmental/Water, Extraction, FTIR, Petroleum
Application Code: Environmental
Methodology Code: Vibrational Spectroscopy
The determination of sulfide and cyanide is of great importance in various samples ranging from environmental, food, chemical, pharmaceutical, food to clinical samples. Due to extremely high toxicity, many worldwide regulatory organizations set their maximum contaminant levels for cyanide and sulfide at very low concentrations. To determine the concentration of cyanide and sulfide accurately and precisely at single low ppb, it is required to develop a reliable analytical method with a limit of detection at sub-ppb levels and free from matrix interferences.

In this paper, we discuss methods in which sulfide and cyanide are separated by using anion-exchange columns and detected by pulsed amperometric detection (PAD) with a thin-film disposable silver working electrode. The advantages of a new separation method using Thermo Scientific Dionex IonPac® AS7 columns and eluents containing oxalate in combination with low concentration of hydroxide and ethylenediamine (EDA) are presented. The possible interference of other species, such as halogens, thiocyanate, thiosulfate and transition metals are discussed and evaluated. Also discussed is the analytical performance of the PAD detection method using the disposable silver electrodes. The new method provides detection limits of 0.1 ppb for sulfide and 0.2 ppb cyanide using a 25 µl injection volume. Calibration plots for sulfide and cyanide are linear from their respective detection limits up to 1,000 ppb. Reproducible long-term performance is demonstrated using repetitive injections of low concentration of sulfide and cyanide.

Abstract Text

Keywords: Electrochemistry, Environmental Analysis, HPLC Detection, Ion Chromatography
Application Code: Environmental
Methodology Code: Electrochemistry
Global concern is growing over the amount of pollution in the atmosphere and the effect on human health. A major source of pollution is particulate matter (PM), solid or liquid particles suspended in the atmosphere. They have the potential for causing health problems in humans due to inhalation of the particles. The smaller the particles, the further they can get into the respiratory system. PM2.5 (particles less than 2.5 micrometers in size) is of particular concern and has been the subject of many health studies linking it to respiratory diseases and increased occurrences of lung cancer. The major sources of PM2.5 are industrial combustion, road transport (from diesel emissions), burning of fossil fuels and small-scale waste burning.

The reference method for measuring PM2.5 is a manual gravimetric method. Samples of PM are taken by pumping ambient air through a size-selective inlet followed by a filter. The concentration of PM is determined by measuring the change in mass of the filter before and after the sampling, and combining the result with the volume of air. Although this method is capable of measuring the total amount of PM2.5 it is not capable of identifying the chemical identity of the materials.

This paper describes the use of the technique of infrared imaging for the detection, identification and quantitation of the different chemical species present in the PM2.5, providing significant information in determining the source of the particulate matter.
Session Title: Environment - Non-Metals and Particulate Determination

Abstract Title: Measuring PM1, PM2.5 and PM10 in Air Quality Networks

Primary Author: John Saffell

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Co-Author(s): Paul Kaye, Paul Williams, Roderic Jones

Abstract Text

Wireless air quality networks have become more affordable in the last years. Low cost wireless technology has been matched with low cost gas sensors that are proving reliable at the ppb concentration levels - recent urban air quality networks have proven that the gas sensors have the required stability, selectivity and sensitivity. The missing requirement was a low cost particle monitor to report on PM1, PM 2.5 and PM10.

An Optical Particle counter (OPC) using a laser source has been used for many years in atmospheric chemistry studies of aerosols and particulates in the stratosphere and troposphere. This design was modified for the Heathrow project and recently commercialised for use in urban air quality networks such as the CITISense European project.

We discuss here results from recent urban air projects and laboratory validation of the OPC. Laboratory validation at Manchester and co-location with AQMs at Cambridge provide us with quantifiable limits on PM and particle size distribution errors. Errors as particle count is translated into particle mass and errors in PM1 calculations will also be discussed.

Correlation of particulate results with CO, NO, NO2 and O3 concentrations gives more information than from any single or coupled measurements, with the fast time resolution telling more about local and background pollutants.

Keywords: Aerosols/Particulates, Air, Environmental/Air, Particle Size and Distribution

Application Code: Environmental

Methodology Code: Integrated Sensor Systems
This project involves constructing and characterizing a fluorescence spectrometer. An average fluorescence spectrometer consists of a light source, excitation monochromator, sample holder, emission monochromator, detector, and a computer and processor. This device is designed to be smaller and portable by eliminating some components from a commercial design. The schematic this fluorescence spectrometer includes a wavelength specific ultra violet LED, a 1mm inner diameter capillary as the sample holder, a wavelength specific photodiode as the detector, and microcomputer as the processor. This set up was characterized using a set of quinine standards. As this setup is wavelength specific, future plans include designing the instrument in such a way that both the light source and detector can be interchangeable or allowing for several sources/detectors to be available in a near simultaneous fashion. This would allow for the possible analysis of other controlled substances to be used for forensic field work, the analysis chlorophyll to be used for biology field work, the ability to monitor protein-protein interactions, and the analysis of various samples in the environment. Future plans also include using an iPhone/iPad application as the computer and processor in order to make the instrument completely wireless.

Keywords: Bioanalytical, Biospectroscopy, Fluorescence, Spectroscopy
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Cerebral Ascorbic acid (AA) detection has become a hot topic in the biological and analytical sciences since AA not only act as a water soluble organic antioxidant protecting oxidative stress but also involve in enzymatic step as a cofactor and is a specific electron donor for enzymes during carnitine biosynthesis, norepinephrine biosynthesis, amidation of peptide hormones, and tyrosine metabolism. Many elegant methods that have been developed for detection of AA including.

Compared with well-established HPLC or GC-MS assay, enzymatic method, capillary electrophoresis and electrochemistry approaches, fluorescence probes sensing ones have attracted rising attention due to their considerable research space and intrinsic advantages such as high sensitivity and selectivity and direct monitoring in live cells, tissues, and animals. Herein, we report a novel and efficient fluorescence probes synthesized by tris (tris(hydroxymethyl)aminomethane)-derived carbon dots (CDs)-modified hexagonal CoOOH nanoflakes (Tris-derived CDs-CoOOH) for monitoring of cerebral AA. The as-prepared Tris-derived CDs as the signal output are fabricated by one-step pyrolysis strategy, then hybridized with CoOOH nanoflakes to form the fluorescence probe Tris-derived CDs-CoOOH. The luminescence of the Tris-derived CDs can be efficiently quenched by CoOOH via Förster resonance energy transfer (FRET) and then by specific redox reaction between CoOOH with AA making high selective to detect AA. The Tris-derived CDs-CoOOH probe show a linear range from 100 nM to 20 µM with the detection limit 10 nM and such high sensitivity makes it applicable for determination of cerebral AA in a rat brain.
Whooping cough is highly contagious. Bordetella pertussis (B. pertussis), the pathogen that causes whooping cough and is considered life threatening to young children, has been at the forefront of the nation’s attention due to the recent outbreaks. Current diagnostic methods for B. pertussis are slow (e.g. bacterial culture), or require expensive and bulky equipment (e.g. qPCR). Therefore, we developed a low-cost PDMS/paper hybrid microfluidic biochip (Figure 1) integrated with loop-mediated isothermal amplification (LAMP) for the rapid, sensitive and instrument-free diagnosis of B. pertussis. Paper placed in each LAMP zone of the hybrid biochip serves as a 3D substrate for preloading and protecting LAMP primers for the subsequent LAMP reaction. Based on the recovered fluorescence of calcein, the fluorescence indicator of LAMP detection, visual detection has been successfully achieved just by using a portable UV light pen. An instrument-free direct pathogen detection approach has been achieved avoiding the use of centrifuge or laborious sample preparation processes. The limit of detection of B. pertussis is about 5 copies per LMAP zone within 1 hour. This low-cost hybrid microfluidic biochip provides a simple but sensitive approach for rapid B. pertussis diagnosis, which has a great potential for point of care detection in resource-limited settings. Financial supports from the NIH and UTEP MRAP are gratefully acknowledged.
Water-soluble conjugated polymers with unique structure have attracted much attention because of their good light-harvesting ability and signal amplification properties, which exhibits the broad applications in biosensor, diagnosis of pathogenic microorganisms and tumor cells, detection of disease-related biomarkers, cell-imaging, drug screening and delivery, photodynamic therapy, etc. Recently, we have devoted ourselves to the design and synthesis of water-soluble conjugated polymers and their applications in biosensors and biochemical analysis. A series of conjugated polymers have been synthesized, such as neutral water-soluble conjugated polymers PPP-OR10, cationic conjugated polymers PFPB, etc. The new biosensing mechanisms have been studied, and new analytical methods have been investigated for detection of enzyme activity, including adenosine deaminase, alpha-chymotrypsin, restriction endonucleases, etc. as well as real-time monitoring of the cleavage of ssDNA by S1 nuclease and phosphorylation of glucose by hexokinase. Our results indicated that these methods are sensitive, cost-effective, simple and rapid without any excess operation, such as separation, denaturation, further reaction, washing steps, etc. Furthermore, the studies are contributed to the WSCPs-based biosensor development, and provide new platforms for biosensors. In future, it is still promising to explore more novel applications by designing and synthesizing novel WSCPs.

Keywords: Biosensors, Enzyme Assays, Fluorescence, Polymers & Plastics

Application Code: Bioanalytical

Methodology Code: Fluorescence/Luminescence
Fluorescence/Luminescence Bioanalytical Applications

**pH Switchable, Charge Dependent Transport in a Weak Polyelectrolyte Multilayer**

Multilayers composed of weak polyelectrolytes (PEMs) exhibit tunable properties making them ideal for use in applications such as drug uptake and selective delivery, separations, and water purification. In order to optimize devices for these applications, an understanding of mass and charge transport in PEMs is required. We have directly measured the interaction of monovalent ions with a multilayer of poly(acrylic acid) and poly(allylamine hydrochloride) and found it to be multimodal, pH tunable and charge dependent. Fluorescence microscopy and single molecule tracking were used to determine the presence of two primary transport modes: 1) adsorption, characterized by periods of immobilization in a subresolution region, and 2) diffusion trajectories characteristic of hopping ($D \sim 10^{-9}$ cm$^2$/s) likely due to intermittent surface interactions. Radius of gyration evolution analysis was used along with simulated trajectories to demonstrate the coexistence of the two transport modes in the same single molecule trajectories. For both anionic and cationic probe molecules, the hopping transport mode could be reversibly switched on and off by changing the solution pH. These results confirm the previously suggested hopping mechanism for the interaction of charged molecules with PEMs and offer insight into the role of electrostatics and nanoscale tunability of transport in weak polyelectrolyte multilayers. This work was funded by the National Science Foundation (CBET-1134417, CHE-1151647, and 1333651), the National Institutes of Health (GM94246-01A1), and the Welch Foundation (C-1787).

**Keywords:** Adsorption, Fluorescence, Microscopy, Polymers & Plastics

**Application Code:** Materials Science

**Methodology Code:** Fluorescence/Luminescence
To self-assemble one-dimensional DNA building blocks into two- and three-dimensional nanostructures via DNA/RNA nanotechnology has led to broad applications in bioimaging, basic biological mechanism studies, disease diagnosis, and drug delivery. However, the cellular uptake of most nucleic acid nanostructures is dependent on passive delivery or the enhanced permeability and retention effect, which may not be suitable for certain types of cancers, especially for treatment in vivo. To meet this need, we have constructed a multifunctional aptamer-based DNA nanoassembly (AptNA) for targeted cancer therapy. In particular, we first designed various Y-shaped functional DNA domains through predesigned base pair hybridization, including targeting aptamers, intercalated anticancer drugs, and therapeutic antisense oligonucleotides. Then these functional DNA domains were linked to an X-shaped DNA core connector, termed a building unit, through the complementary sequences in the arms of functional domains and connector. Finally, hundreds (100–200) of these basic building units with 5'-modification of acrydite groups were further photo-cross-linked into a multifunctional and programmable aptamer-based nanoassembly structure able to take advantage of facile modular design and assembly, high programmability, excellent biostability and biocompatibility, as well as selective recognition and transportation. With these properties, AptNAs were demonstrated to have specific cytotoxic effect against leukemia cells. Moreover, the incorporation of therapeutic antisense oligonucleotides resulted in the inhibition of P-gp expression (a drug eflux pump to increase excretion of anticancer drugs) as well as a decrease in drug resistance. Therefore, these multifunctional and programmable aptamer-based DNA nanoassemblies show promise as candidates for targeted drug delivery and cancer therapy.
Biochemists are investigating detection methods for biological samples such as protein and DNA. In this study, we have designed and synthesized three fluorescent molecular probes based on dansyl fluorophores; i) fluorescent molecule possessing both Zn(II) complexes and dansyl moieties for total protein electrophoretic analysis (Probe 1), ii) fluorescent oligonucleotides (ONTs) prepared from nonnucleoside amidite block of dansyl fluorophore to detect target DNA (Probe 2), iii) fluorescent reagent using FRET between cyanopyranyl group and dansyl fluorophore for the detection of proteins (Probe 3) to reduce the background fluorescence from dansyl group and achieve highly sensitive detection.

Probe 1, which produced strong fluorescence on the addition of proteins, allowed 1-step protein staining (SDS-PAGE Staining Detection) and shortened the operating time (35 min) with high sensitivity (limit of detection: 1.0 ng or less). Fluorescence intensities of Probe 2 specifically increased by the presence of adjacent guanosine residues but, significantly reduced in a dansyl-flipping duplex with good linear relationship between fluorescence intensity and target DNA concentrations. These changes were caused by solvatochromism effect due to the number of guanine which is hydrophobic functional group and the external environment change around dansyl residue.

Fluorescence quantum yield of Probe 3 itself ([\Phi] < 0.001) was about 100 times lower than those of Probe 1 and Probe 2 due to the fluorescence quenching of dansyl group by cyanopyranyl group, whereas fluorescence intensity of Probe 3 increased upon binding to BSA ([\Phi] = 0.49). These phenomena demonstrated the highly sensitive detection of proteins (limit of detection: 0.1ng on the electrophoretic gel).

These results proved that above three fluorescent probes could be useful for a convenient and highly sensitive detections of protein and DNA in biochemical and medical fields.

Keywords: Biosensors, Fluorescence, Nucleic Acids, Protein
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
DNA micelle flares are sphere-shaped DNA-lipid structures formed by hydrophobic interactions between the lipid tails coupled to single DNA strands. Compared to other sphere-shaped nucleic acids, DNA micelle flares have high cellular permeabilities and low critical micelle concentrations, making them potential carriers for cancer drugs. For this purpose, the particles must remain stable under various conditions dictated by the drug, such as low pH and in organic solvents. In this work, the basic properties of DNA micelles have been studied using fluorescence, static light scattering and UV-Vis detection for systematic investigations of enzyme resistance, complementary DNA binding affinity and micellar melting temperature. Additional studies have included measurements and comparisons of aggregation number and critical micelle concentration of micelles of different lengths. Results have shown that micelles have better enzyme resistance towards DNase I and Exo III compared to molecular beacons containing the same base pairs. The critical micelle concentration is below 5nM for all the micelles being tested and the aggregation number remains stable for micelles of different DNA lengths. The stability of DNA micelles in low pH conditions has also been verified.

**Keywords:** Analysis, Characterization, Fluorescence, Nucleic Acids

**Application Code:** Biomedical

**Methodology Code:** Fluorescence/Luminescence
Abstract Text

Determination of blood alcohol concentration (BAC) is one of the most common analyses performed within forensic laboratories. This analysis most commonly utilizes headspace sampling, followed by gas chromatography combined with flame ionization detection (GC-FID). Amongst the many instrumental parameters, helium has historically been the chosen carrier gas, largely based on convenience and the potential safety issues associated with Hydrogen. According to the Van Deemter Equation, hydrogen has proven to reduce run times due to its high diffusivity and high optimal linear velocity. A potential issue due to the emerging helium shortage, and increasing cost, has led to the evaluation of using hydrogen supplied through a generator system for application to this routine analysis.

The goal of this research is to investigate the differences in chromatography and efficiency of BAC determination when either helium or hydrogen is used as a carrier gas. Quality by design (QBD) will be implemented in order to determine the appropriate corresponding BAC instrumental parameters when converting to hydrogen as a carrier gas. This presentation will discuss the differences in efficiency between helium and hydrogen as applied to the Van Deemter equation, as well as their safety within this application. Additionally, resolution and overall chromatographic performance will be thoroughly discussed. Finally, the degree of uncertainty at the common threshold of 0.08 g/dL and method detection limits will be reported in order to determine a reporting threshold.

Keywords: Forensic Chemistry, Gas Chromatography, Method Development
Application Code: Pharmaceutical
Methodology Code: Gas Chromatography
Cellular fatty acid profiling is a valuable tool for identifying the species/strain of bacteria within a forensic sample as well as the culturing conditions used in the laboratory. However, traditional fatty acid analysis involves lysing the organism to extract membrane phospholipids. This often prevents other types of chemical or genetic analyses from being performed on the same sample. The goal of this study was to investigate extracellular fatty acid profiles as a forensic signature for Bacillus spores. Extracellular reservoirs can be extracted from the cell non-destructively thereby preserving the rest of the cell for other techniques. To test this, six strains of Bacillus organisms (Bacillus cereus T-strain, Bacillus cereus 14579, Bacillus anthracis 690, Bacillus anthracis 0517, Bacillus thuringiensis HD1 and Bacillus thuringiensis HD522) were cultured in three separate medium formulations: milk-digest, meat-digest, and yeast. Fatty acids from the cell surface were extracted in acetone, converted into fatty acid methyl esters, and profiled by GC-FID.

Results showed that the types and relative abundance of fatty acid structures on the spore surface could be used to determine the species/strain and the growth medium used to prepare an unknown Bacillus sample. Specific fatty acids were also unique to certain strains, aiding taxonomic characterization (e.g., 16:1\(\text{cis-9}\) in BtHD522). Distinct variation in 17:0anteiso, 14:0iso, and 17:1iso markers was indicative of growth medium. Discriminant function analysis using these variables provided clear separation of nutrient source for each strain. These non-destructive methods could provide novel data streams during a forensic investigation while permitting further sample analyses.

Keywords: Biological Samples, Characterization, Forensics, GC
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography
The US Environmental Protection Agency Method 8095 gives guidelines for the use of gas chromatography with electron capture detection (GC-ECD) for explosive residue analysis. There have since been advances that may increase sensitivity or detection. Previous gas chromatographic studies on explosives analysis focus on variables like column chemistry, inlet temperature, injection methods, & detector variability[1,2]. Amid these studies are none focused on effects of surface deactivation, inlet liner geometry or guard column length for the gas chromatographic explosive analysis. While studies mention using deactivated inlets the chemistry of this surface & inlet liner geometry hasn’t been studied. Thus, a study on the combination of inlet geometry, stationary phase chemistry & guard column length is useful. The goal is to find more efficient chromatography parameters for explosives analysis. The main focus is optimization of deactivation chemistry applied to inlet liner geometry & columns. Determination of the ideal method for gas chromatographic analysis of explosives with improved sensitivity, especially with more thermally unstable analytes will be discussed. Currently, ion mobility spectrometry is used but it has high false-positive rates[3]. Since TOFMS can be a highly selective method, we will discuss if LC-TOFMS & DSA-TOFMS is more selective & feasible as a reliable explosives testing device for law enforcement.


Keywords: Chromatography, Detection, Gas Chromatography, Liquid Chromatography/Mass Spectroscopy
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography
Petroleum and petroleum-based products are ubiquitous and include, gasoline, diesel, kerosene, jet fuels, oils and lubricants – among others. Effective monitoring, measurement and analysis of these products are essential. One compound which has been difficult to determine in petroleum products is water. The water content affects the refining, performance and overall quality of the product. It also is considered a parameter that affects the purchasing/sale of these products. The complex matrix leads to many side reactions with traditional methods for water determination. For instance, in the Karl Fischer titration, the presence of sulfur containing compounds leads to an over estimation of water content. Other methods like loss on drying are difficult since it determines the mass of all volatile components and is therefore not specific to water. We have developed a sensitive, accurate headspace gas chromatographic method utilizing the novel dielectric barrier ionization detector for the determination and quantification of water. The water is chromatographically separated from any volatile compounds alleviating problems associated with complex matrices and other volatile components. This leads to a method that is rapid, precise and not affected by side reactions or matrix effects.

Keywords: Gas Chromatography, Headspace, Petroleum, Water
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography
Fossil fuels such as diesel fuel and jet fuel are some of the most important sources of energy in today's society. Both the performance and emission properties of these types of fuels are influenced by their group-type composition (i.e. saturates, mono-, di-, tri- and polycyclic aromatics). Since there is such a widespread use of diesel fuels government limits on the poly aromatic hydrocarbon content of diesel fuels are becoming increasingly stringent. ASTM: D5186-03 is a test method that determines the aromatic and polynuclear aromatic content of diesel fuels and aviation turbine fuels by supercritical fluid chromatorgraphy (SFC).

However, access to SFC instrumentation is increasingly difficult to obtain, while gas chromatographic (GC) instrumentation is commonplace. Furthermore, the “bio” component of biodiesel does not necessarily elute quantitatively from the columns used in the SFC method. Previously, GC separations requiring multidimensional separations and/or mass spectrometric detectors (that give non-uniform responses) have been used to achieve group-type separations. The recent introduction of ionic liquid (IL) columns for use in GC presents an opportunity for the development of a conventional GC separation that may deliver a suitable group-type separation for certain petroleum samples. Group-type separation through the use of IL columns in conjunction with a GC-UV-FID to will be explored in this research. Initial studies demonstrate the potential of GC-UV-FID to differentiate the few overlapping aromatic and saturate responses that remain for diesel-range fuels.

Keywords: Capillary GC, Fuels\Energy\Petrochemical, PAH, Spectrophotometry
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography
The petroleum industry has a need to enhance the accuracy of the boiling point distribution received from method ASTM D7169. The accuracy is compromised by solvent interference in the C4 to C8 boiling point range. The proposal from ASTM is to report the distribution from C9 minus from a DHA analysis which separates the carbon disulfide (CS2) peak on one gas chromatograph (GC), and merge the results received from a classical D7169 for C9 plus from another GC which requires two GCs.

This paper will discuss innovative techniques demonstrating the ability to meet the goal of improved accuracy on one GC instead of two reducing costs and bench space. It will save time by reducing sample preparation and data processing enhancing productivity.

With the use of multi-dimensional GC, improvements in columns, and innovative capability, a method for the C1 to C100 analysis of a crude oil was developed with enhanced accuracy on one GC.

Additional improvements are the ability to attain more information from crude other than boiling point distribution, such as:
- Quantitate n-paraffins below C8
- Improve wax profile
- Separate bio-markers such as pristane and phytane
- Determine crude quality
- Resolve Benzene and Toluene for quantitation

**Keywords:** Capillary GC, Fuels\Energy\Petrochemical, Petrochemical, Petroleum

**Application Code:** Fuels, Energy and Petrochemical

**Methodology Code:** Gas Chromatography
As LPG is transported through pipelines, hoses, and tanks it comes into contact with many contaminants, potentially resulting in a heavy residue in the LPG. This residue, when left unchecked can harm seals, valves, and even engines. Understanding the composition of this residue is important to determine the source of problems. In addition to LPG, there are many other pressurized gases that require investigation.

This presentation discusses an accurate and precise technique, answering the question is there contamination, and if so, how much of this contamination is present.

This presentation will demonstrate the ability to analyze for the hydrocarbon range between C6 through C44 in pressurized gas using sorbent tube technology, thermal desorption, and gas chromatography. This ability to analyze this broad hydrocarbon range in gases is advantageous in many petrochemical and environmental matrices. Many of these matrices will be discussed in addition to LPG.

**Keywords:** Fuels, Energy, Petrochemical, Gas Chromatography, Hydrocarbons, Petrochemical

**Application Code:** Fuels, Energy and Petrochemical

**Methodology Code:** Gas Chromatography
## Sampling and Sample Preparation - SPME; Clinical, Forensic

**Utility of BioSPME for Micro Extraction of Biological Fluids**

There has been a growing trend in bioanalysis toward the utilization of micro extraction techniques for sample isolation and transportation. Not only are there ethical advantages for test subjects, but there is also an interest in cost reduction associated with sampling. This study explores the utility of biocompatible solid phase microextraction (BioSPME) as a viable means for sample preparation in bioanalysis. A model set of compounds is used to explore the utility of BioSPME across a range of biological matrices. The unique feature of the BioSPME technique is that it enables direct analysis of biological samples without the need for protein precipitation, centrifugation or digestion. The BioSPME technique allows for isolation of target analytes while minimizing coextraction of sample matrix allowing for more sensitive and robust bioanalysis.

**Keywords:** Clinical Chemistry, Sample Preparation, SPME

**Abstract Text**

There has been a growing trend in bioanalysis toward the utilization of micro extraction techniques for sample isolation and transportation. Not only are there ethical advantages for test subjects, but there is also an interest in cost reduction associated with sampling. This study explores the utility of biocompatible solid phase microextraction (BioSPME) as a viable means for sample preparation in bioanalysis. A model set of compounds is used to explore the utility of BioSPME across a range of biological matrices. The unique feature of the BioSPME technique is that it enables direct analysis of biological samples without the need for protein precipitation, centrifugation or digestion. The BioSPME technique allows for isolation of target analytes while minimizing coextraction of sample matrix allowing for more sensitive and robust bioanalysis.

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**Session Title**
Sampling and Sample Preparation - SPME; Clinical, Forensic

**Abstract Title**
Utility of BioSPME for Micro Extraction of Biological Fluids

**Date:** Sunday, March 08, 2015 - Afternoon
**Time:** 01:30 PM
**Room:** 267

## Abstract # 220-1

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**Abstract Text**

There has been a growing trend in bioanalysis toward the utilization of micro extraction techniques for sample isolation and transportation. Not only are there ethical advantages for test subjects, but there is also an interest in cost reduction associated with sampling. This study explores the utility of biocompatible solid phase microextraction (BioSPME) as a viable means for sample preparation in bioanalysis. A model set of compounds is used to explore the utility of BioSPME across a range of biological matrices. The unique feature of the BioSPME technique is that it enables direct analysis of biological samples without the need for protein precipitation, centrifugation or digestion. The BioSPME technique allows for isolation of target analytes while minimizing coextraction of sample matrix allowing for more sensitive and robust bioanalysis.

**Keywords:** Clinical Chemistry, Sample Preparation, SPME

**Application Code:** Other

**Methodology Code:** Sampling and Sample Preparation
The average age of many large power transformers around the globe is reaching 40 or more years. Due to cost and today’s growing demand for power, many countries are choosing to maintain rather than replace transformers. Maintaining power transformers involves routine testing of the transformer oil to monitor the furan levels present, with the intent to provide assurance of both transformer safety and power supply reliability to communities across the globe. Unexpected failures of power transformers can lead to significant costs - a replacement transformer, environmental cleanup from the failure, as well as potential financial penalties for not delivering power to communities. With these financial and safety factors in mind, testing of aging power transformers is increasing significantly using the traditional manual ASTM D5837 solid phase extraction procedure of the sampled transformer oil. Frequency and number of samples tested is growing, leaving many power companies and contract laboratories searching for new automated solutions that use the ASTM procedure to offer simplicity and efficiency versus hiring and training more laboratory chemists. This application presents data to compare automated and manual solid phase extraction ASTM D5837 testing procedures to offer a simplified and more efficient approach for workflow, accuracy, precision, and operator time.

Keywords: Energy, Fuels\Energy\Petrochemical, Petroleum, Solid Phase Extraction
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Sampling and Sample Preparation
A new line of fibers that contain an overcoating-layer of a proprietary PDMS over an adsorbent fiber coating has been developed. The primary advantage of adsorbent based fiber coatings is that the porous materials retain analytes more tightly than absorbent type fiber coatings. This analyte retention advantage may become a disadvantage if the fiber has to be immersed in a complex matrix sample. The properties of adsorbent coatings that retain analytes tightly also may tightly retain nonvolatile components in the matrix. This can result in fouling of the fiber coating and shorten the lifespan of the fiber. By applying a layer of PDMS coating over the adsorbent coating, many of the large components in the matrix are repelled, while the smaller analytes of interest can pass through the outer PDMS layer and be retained by the adsorbent.

This presentation will compare the lifespan of overcoated fibers to adsorbent fiber coatings without the PDMS overcoat layer out of complex matrices such as fruit juices. The effect of the matrix on the lifespan of the coatings will be demonstrated with pictures and data. The effectiveness of the overcoated fibers from a variety of matrices will be presented. Because the PDMS overcoating slightly changes analyte uptake kinetics, data will be shown that compare the differences. Also, we will share some tips on how to use the overcoated fibers more effectively.

Keywords: Gas Chromatography, SPME
Application Code: Other
Methodology Code: Sampling and Sample Preparation
QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) is an extraction technique that combines a liquid-liquid microextraction and a dispersive solid phase extraction cleanup. This technique has been used primarily for the extraction of pesticides from food products and applied to a wide array of pesticides in a multitude of food matrices including fruits, vegetables, teas, fatty food products, and more. This presentation will discuss an overview of QuEChERS and its methodology as it pertains to the extraction of glucocorticoid steroids such as prednisolone, hydrocortisone, dexamethasone, and methylprednisolone from water and herbal medicinal products (HMPs). These steroids are frequently used in adulteration of HMPs to speed the healing process; thus it is necessary to develop an analytical method to detect the presence of these illegal adulterants. Both gas chromatography triple quadrupole (GC/MS/MS) and two-dimensional gas chromatography time of flight mass spectrometry (GCxGC-ToFMS) were used for the analysis of these steroids once extracted from water and herbal medicines using QuEChERS. The optimization of both instruments, such as MRM parameters for GC-MS/MS and modulator parameters for GCxGC-ToFMS will be discussed as well as optimization of the QuEChERS extraction for these steroids.

Keywords: Forensics, GC-MS, Sample Preparation
Application Code: Clinical/Toxicology
Methodology Code: Sampling and Sample Preparation
Solid phase microextraction (SPME) is a fast, easy, and reliable technique used for extraction of analytes from various matrices such as waste water, air, etc. The extraction phase for an SPME fiber can be either a solid or a liquid. Liquid coatings such as PDMS yield good linear ranges and show no competitive displacement effects, but the extraction process is slow. On the other hand, solid coatings such as DVB-PDMS offer faster extractions and better selectivity, but exhibit competitive displacement effects. Recently, our group has developed a novel, sputtered SPME coating. These coatings are thin (ca. 1 µm), porous, and do not require any adhesive for attachment to the substrate. The resulting fibers have yielded good extraction efficiencies for mixtures of alkanes and alcohols. We expect these novel fibers to be robust and compatible with numerous organic solvents. With regards to thermal stability, the fibers will be exposed to different temperatures: 260, 280, 300, and 320 °C for a specific period of time and their extraction performance will be evaluated. The longevity of the fibers will be determined by monitoring the extraction efficiencies of the fiber after each extraction at a specific temperature until there is a decrease in the extraction capability of the fiber.

Keywords: GC, Headspace, SPME
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Since its inception in 2007, the HybridSPE technology has demonstrated to be an instrumental platform for mitigating ion suppression/matrix interference in LC/MS applications. Peer review publications on this technology have stemmed across a broad range of disciplines from clinical applications to various “omics” fields. The utility of the technology stems from the ability harness the selective Lewis Acid/Base affinity of the Zirconia-Si particles towards electron rich phosphate moieties for both reduction of biological matrix interference and targeted isolation of phospholipids. This presentation serves as an overview of peer reviewed journal publications where the HybridSPE platform has been implemented in various disciplines for targeted isolation, enrichment, depletion and profiling of phospholipids.

Abstract Text

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Keywords: Clinical Chemistry, Forensics, Sample Preparation
Application Code: Other
Methodology Code: Sampling and Sample Preparation
Sampling and Sample Preparation - SPME; Clinical, Forensic

Microwave Sample Preparation of Infant Formula and Nutritional Supplements

Dietary supplements are a rapidly growing segment of the food and beverage industry around the world but particularly in the United States. The ingredients which make up the composition of these supplements are sourced from around the globe making testing of these products of critical importance. A new USP method 2232 is in the process of final approval which will standardize the testing of these materials. Closed vessel digestion is listed as a way to prepare samples using Method 2232 and microwave digestion provides a rapid preparation step as compared to other techniques. Limited sample size has prevented its use for some applications especially for infant formula. We will show the possibility of preparing a wide variety of supplements and infant formula samples in a microwave system using sample sizes of up to two grams. We will illustrate the step wise approach required to prepare these samples as well as show recovery data for reference materials as well as spike recoveries for both volatile and non volatile elements.

Keywords: Atomic Emission Spectroscopy, Food Contaminants, Microwave, Sample Preparation
Application Code: Food Safety
Methodology Code: Atomic Spectroscopy/Elemental Analysis
A drug delivery system or a formulation typically consists of several ingredients, the compatibility of which can influence its biological activity and its shelf life. Therefore, investigation of interactions between active pharmaceutical ingredients (APIs) and excipients is important during the development process of new drugs to achieve a finally acceptable drug product stability. Due to its sensitivity to changes in composition and structure, differential scanning calorimetry (DSC) is a fast and reliable method to detect incompatibilities; with thermogravimetric analysis (TGA) it is possible to quantify volatile products and TGA-FTIR (thermogravimetric analysis combined with Fourier transform infrared spectroscopy) allows identification of the evaporating gases. These methods were used to investigate binary mixtures of ibuprofen – an analgesic – and magnesium stearate, a widely used anti-caking agent.
The interior of cells consists of a heterogeneous mixture of macromolecules that are tens to hundreds of times more concentrated than the dilute conditions used in most biophysical studies. Both theoretical and experimental evidence suggests that enzymes behave differently under such conditions resulting in changes to stability, diffusion, aggregation, association, and ultimately function. To better understand how macromolecular crowding affects enzyme kinetics, we used spectrophotometric assays to measure the Michaelis-Menten parameters for the enzyme yeast alcohol dehydrogenase (YADH) in the absence and presence of crowding agents. These crowding agents vary from inert sugar polymers, such as dextran, to charged proteins, such as BSA. Our results show that crowding enhances the binding affinity of YADH for both of its substrates while decreasing the maximum reaction rate. Furthermore, the extent of these effects depends on the dextran concentration but not the size of the polymers.

Since diffusion plays such an important role in enzyme kinetics, we have also used electrochemical methods to measure diffusion coefficients of small molecules in crowded solutions. These electrochemical measurements both complement the kinetic studies and offer a more general insight into diffusion in crowded media. Again, the concentration of the crowding agent more than its size plays a role in determining the diffusion coefficient. Comparing these values to the solution viscosities revealed that dextran solutions do not obey Stokes-Einstein. Thus, this work provides insight about protein movement within the densely packed cellular environment.
For properly maintaining the operation of the waste water treating facilities, in-situ analysis of the water quality is very important. Ammonium-nitrogen has been used as an index of the water pollution because it is formed when the organic compound in water is corrupted and decomposed.

Ammonium-nitrogen in the water treating process is oxidized to nitrite-nitrogen (NO2), then to nitrate-nitrogen (NO3). Thus the concentration of ammonium-nitrogen has been utilized as an index to show the nitrification process condition in the biological reactors in the sewage treatment plants and/or septic tanks. Meanwhile, ammonium-nitrogen is also used as an effective water quality index to determine the chlorine injection rate in the disinfection process by chlorine. In this way, measurement of ammonium-nitrogen is commonly done for daily monitoring and diagnosing the condition of waste water plants.

In this report, we describe the development of a new ammonia analyzer using a coulometric titration method. We will show that this new method is considerably faster and easier to use while the data shows very high correlation when compared to conventional methods. The measurement principle will also be described.
Extended HPLC system operation during overnight or weekend operation requires either a sufficient volume of solvent or operator intervention to prevent the system from running dry.

In this report, we describe the development of a new sonic level sensor that fits into the HPLC solvent reservoir cap. The sensor employs sonic wave technology that can monitor the level of solvent in a maximum of four solvent reservoirs. We will describe in detail the real-time monitoring of the solvent level and will show other safety features like shutting down the HPLC pump should solvent levels become critically low.

**Keywords:** HPLC, Integrated Sensor Systems, Laboratory Automation, Solvent

**Application Code:** Pharmaceutical

**Methodology Code:** Sensors
New Developments in Analytical Instrumentation and Software

Evaluation of the Mutagenic Activity Due to Heavy Metals of Water Collected From the Cauca River in the City of Cali, Columbia by Using the Ames Test

The Cauca River is an important hydric and economic resource for the Republic of Colombia and especially for the City of Cali because it provides the water for human consumption. Unfortunately, there are several sources of contamination from agricultural, industrial and domestic residual waters. This has resulted in widespread heavy metals contamination and it is well known that these metals can be introduced in the food chain by several species of native fish.

In this work the mutagenic activity of the Cauca River water due to the presence of heavy metals (Hg, Pb, Cr and Cd) was evaluated in the urban area of the city of Cali by the Ames test. Samples of crude water were taken at five different places during dry and rainy seasons. The metals were extracted from water samples using the Amberlite XAD-16 resin. The concentrations of heavy metals were measured by Atomic Absorption Spectrophotometry and mutagenicity was evaluated using the TA98 and TA100 strains of Salmonella typhimurium with and without the S9 enzymatic activator to simulate the effect of contaminants in the liver of mammals.

The results showed mutagenic activity with Mutagenicity Indices (MI > 2.0) in three of the five points evaluated: The El Hormiguero bridge (MI = 3.6), the mouth of southern collector channel (MI = 2.9) and the mouth of the Cali River (MI = 2.7). These sampling points in turn presented the highest total concentrations of heavy metals in the extracts. The determination and analysis of spatio-temporal variation of the mutagenic effects obtained was done by conducting multifactorial variance analysis of MI, finding that the effect of sampling season (dry o rainy season) contributes significantly to the variability of MI unlike the sampling points.

Keywords: Atomic Absorption, Environmental Analysis, Environmental/Water, Metals

Application Code: Environmental

Methodology Code: Atomic Spectroscopy/Elemental Analysis
GC-MS with Cold EI is based on interfacing the GC and MS with supersonic molecular beams (SMB) along with electron ionization of vibrationally cold sample compounds in SMB in a fly-through ion source (hence the name Cold EI). The Aviv Analytical 5975-SMB GC-MS with Cold EI provides enhanced molecular ions, significantly extended range of compounds amenable for analysis, much faster analysis and lower limits of detection particularly for difficult to analyze compounds. However, in order to convert GC-MS with Cold EI into ideally suitable solutions for challenging applications we further developed and combined it with several additional enhancement technologies including:

A. A unique low thermal mass fast GC enables sub one minute full analysis cycle time with short standard fused silica capillary columns.
B. Open Probe Fast GC-MS with Cold EI enables the provision of real time analysis with separation and library identification including with minimal or no sample preparation.
C. ChromatoProbe sample introduction device serves as an effective MS probe and as manual thermal desorption unit for dirty samples analysis with minimal or no sample preparation and with enhanced sample integrity.
D. Pulsed Flow Modulation GCxGC was combined with GC-MS with Cold EI for the combination of improved separation with best identification and the elimination of ion source peak tailing and second column peak fronting.
E. Improved sample identification was achieved via TAMI software that provides improved quadrupole mass accuracy and combined with isotope abundance analysis it provides elemental formulae and is automatically linked with the NIST library.

Keywords: Gas Chromatography/Mass Spectrometry, GC-MS, Instrumentation
Application Code: Other
Methodology Code: Gas Chromatography/Mass Spectrometry
Silica-coated core-shell magnetic nanoparticles, having both the properties of magnetic nanoparticles as well as specifically tailored chemical properties, are important for biomedical applications. In this study manganese based magnetic nanoparticles (MNP) were synthesized using micro-emulsion and then used as support for other nonmaterial’s including silica. These nonmaterials were characterized using X-ray Diffraction (XRD) and Scanning Electron Microscope (SEM) and DLS. To demonstrate usability of these nonmaterials, anti-human IgG, was immobilized on the surface of the nanoparticles. The immunological reaction with the human IgG antigen was then carried out on the surface. Detection of the anti-human IgG reaction with the antigen on the manganese based magnetic nanoparticle was followed using electrochemistry. This approach provides an immunosensor with a low antigen detection limit and provides a platform for several applications in protein analysis.
New Developments in Analytical Instrumentation and Software

Inline Dilution for Enhanced Oil Analysis by ICP-OES

While ICP-OES technology has undergone considerable developments on the spectrometer side, challenges related to the sample matrix still remain. For example, oil analysis by ICP-OES can be challenging due to use of organic solvents. When organic solvents are introduced into the plasma the temperature gradient along the axis of the ICP torch increases more significantly. This increase in the temperature gradient can lead to premature failure of the standard quartz ICP torch. Oil analysis also often requires labor intensive sample preparation with volatile organic solvents. In this paper we will present two sample introduction components specifically designed to increases both the speed and accuracy of oil analysis by ICP-OES. An enhanced productivity system eliminates the need for manual dilutions by incorporating three precision syringe drives and a switching valve, allowing for a sample of undiluted oil to be diluted in-line with kerosene and precisely delivered to the ICP spectrometer. A fully ceramic demountable torch, comprised of a sialon outer tube eliminates premature ICP torch failure and improves long-term signal stability.

Keywords: Flow Injection Analysis, ICP, Petrochemical, Sample Introduction
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
A novel simple, direct and selective stability-indicating GC-MS procedure was developed for the determination of the antihistamine drug dimenhydrinate (DMH) in presence of six of its related substances and potential impurities, namely, diphenylmethane, diphenylmethanol, benzophenone, orphenadrine, caffeine and 8-chlorocaffeine. The method involved resolution of the underivatized compounds using a trifluoropropylmethyl polysiloxane (Rtx-200) capillary column and the mass spectrometric detection was carried out in the electron-impact (EI) mode. Excellent baseline separation of DMH and the cited related substances was achieved in less than 15 min. Quantification of the parent drug DMH was based on measuring its peak area. The reliability and analytical performance of the proposed method were statistically validated with respect to linearity, range, precision, accuracy, specificity, robustness, detection and quantification limits. Calibration curve of DMH was linear over the range 50–500 µg/mL with $r^2 = 0.9982$. The proposed method was successfully applied for the assay of DMH in tablets dosage form with recoveries not less than 96.80%.
We report on the application of photomicroscopy for kinetic measurements of the heterogeneous reaction of cinnamyl chloride on indium metal in solvent/water mixes for four different solvents. In some cases, the reaction is predominantly diffusion controlled, for others it is kinetically controlled, and for yet others, it is mixed. For each solvent mix we measure rates of reaction at different convection rates. We simulate the results using a mixed diffusion- and kinetic-control model in which the heterogeneous rate constant and diffusion coefficient are the only variables. We show that the reaction rate increases as the percent water increases, but in most cases depends only slightly on the type of organic solvent. Perhaps more importantly, we show that the modelling of the data allows a wider range of heterogeneous rate constants to be measured than possible with a simple analysis of the data.

Keywords: Electrode Surfaces, Microscopy
Application Code: Other
Methodology Code: Microscopy
Based on the geometrical arrangement of the stationary phase, there are planar- and column chromatographic techniques. Planar chromatography operates with liquid mobile phase. Mobile phase progress takes place as consequence of several types of internal or external forces. Internal force is capillarity what forwards the solvent system (mobile phase) on the dry stationary mobile phase. External forces can be gravity, electric field, centrifugal force and a pump that force flow the mobile phase. The generally used version of planar (thin-layer) chromatography (TLC) utilizes ascending direction of development when mobile phase starts to run on a dry stationary phase. However, the constantly decreasing flow velocity of the mobile phase front is determined by the vapor phase and its saturation. It is the major reason that exerting of external forces of mobile phase progress has widely been suggested and used. Descending mode of development utilizes gravity for development. Supply of mobile phase by centrifugal forces gives an easy way of technical arrangements. Circular form of developments is reached and its application includes both analytical and preparative aims. Decreasing speed of front progress is resulted from circularity. Forced-flow development of planar chromatography is also possible using pump(s). Both circular and linear shape of mobile phase front can be generated using a special chamber called OPLC (overpressured-layer chromatographic set-up). Another way of mobile phase movement is planar electrochromatography. Basic theoretical rules, numerical formulas, advantages versus disadvantages of the various set-ups, and practical solutions will be given together with mode of optimizations.

**Keywords:** Liquid Chromatography, Method Development, Separation Sciences

**Application Code:** Bioanalytical

**Methodology Code:** Liquid Chromatography
Some drugs and other xenobiotics contain either N-, O-or S-methyl group in their chemical structures. Their metabolism can yield desmethylated product together with formaldehyde. Their ADME (absorption-distribution-metabolism-excretion) is generally followed using reversed-phase high performance liquid chromatography (RP-HPLC). So can be done to localize the site of their desmethylation metabolism. The fragment metabolite (here, the formaldehyde) should also be determined in the compartment of question. Rats were intraperitoneally treated with 14C-methyl labeled selegiline (radiolabelled L-deprenyl). Distribution of the radiolabelled drug was followed by autoradiography to indicate organs and tissues of interest. Various tissues were dissected and serum was prepared following 15, 30 and 60 minutes following treatment and radioactivity was determined after dissolving one part of the samples using Soluene. Another part of the dissected tissues was homogenized and the formaldehyde and the deprenyl+methamphetamine contents were determined using RP-HPLC. Quantitative determination of formaldehyde required its derivatization using 2,4-dinitrophenyl hydrazine (DNPH), and the DNPH-formaldehyde adduct was subjected to RP-HPLC. Both ultraviolet absorbance and the radioactivity in the collected fractions were monitored to identify the formaldehyde content metabolically generated from radiolabelled L-deprenyl.

This project has been supported by the Hungarian National Granting Agency (grant No.: OTKA 100155). Animal treatment followed the ethical regulations.
In the case of reversed-phase liquid chromatography (RPLC), aqueous solutions of methanol or acetonitrile are frequently used as the mobile phase solvents. On the other hand, it was hard to use water as the mobile phase in RPLC systems because of the tangle of alkyl ligands on the stationary phase surface. It is required to clarify the chromatographic behavior in the RPLC systems using water as the mobile phase.

Chromatographic behavior in RPLC systems using water as the mobile phase was experimentally studied on the basis of the moment analysis. The values of retention equilibrium constant ([i]K[/i]) and surface diffusion coefficient ([i]D[/i][sub]s[/sub]) were respectively measured as the equilibrium and kinetic parameters for the RPLC systems. Additionally, the temperature dependence of [i]K[/i] and [i]D[/i][sub]s[/sub] was analyzed to determine the heat of retention ([i]Q[/i][sub]st[/sub]) and the activation energy of surface diffusion ([i]E[/i][sub]s[/sub]) by the van’t Hoff and Arrhenius plots, respectively. It was concluded that chromatographic behavior in the RPLC systems using water as the mobile phase was similar to that in the RPLC systems using the mixtures of organic solvents and water.

In this study, the intermolecular interactions between solute molecules and alkyl ligands on the stationary phase was also analyzed by molecular orbital (MO) calculations. The interaction energy estimated by the MO calculations was comparable with the value of [i]Q[/i][sub]st[/sub] experimentally measured. It was demonstrated that computational chemistry was one of useful strategies for the analysis of chromatographic behavior.

**Keywords:** HPLC, Separation Sciences

**Application Code:** Other

**Methodology Code:** Liquid Chromatography
In this interdisciplinary study, Chemistry and Art faculty and students collaborated to analyze, characterize and digitize artifacts from the Earlham College Art Collection. The goal of this project was to better characterize the uses and limitations of handheld spectrometers to obtain chemical information about art objects that can only be analyzed within our Art Collection. A total of 106 Chinese and African ceramic objects were analyzed by handheld INNOV-X alpha 6500 X-Ray Fluorescence spectrometer (hh-XRF) and by Diffuse Reflectance Infra-Red Spectroscopy (DRIFT) using a handheld 4100 Agilent ExoScan Series FTIR analyzer. Principal component analysis (PCA) was performed on all unglazed specimens using the log-scaled concentrations for Zr, Rb, Sr, Ti, Fe and Mn, and was able to differentiate the African objects from the Chinese objects. In the African group (56 objects), PCA using Zr, Fe, Rb and Sr showed six distinct clusters. In the Chinese group (50 objects), PCA using Zr, Rb and Sr also showed six distinct clusters that correlated well with the different time periods. From the Chinese glazed specimens, we were able to identify two distinct types of glazes: one heavy in Ca and one heavy in Pb. The Pb glaze on the Han Dynasty model stove matched that of a vase of uncertain origin, which reclassified the vessel's place of origin. DRIFT analyses were used to estimate the firing temperature of the ceramics objects. The SiO-stretching bands indicated that the West African objects were fired at lower temperatures (500 to 600 °C) than the Chinese objects (600 to 900 °C).

Keywords: Art/Archaeology, Elemental Analysis, FTIR, X-ray Fluorescence
Application Code: Art/Archaeology
Methodology Code: Portable Instruments
Organic residues in archaeological pottery fragments were analyzed using gas chromatography-mass spectrometry (GC-MS) to determine fatty acid ratios (C12-C22), which allows for the identification of food groups. Combined with ethnography and anthropological research, the extracted fatty acid ratios can be useful in determining specific foods and uses of the vessels through the food groups consumed. Pottery sherds from the Pacific Northwest were subjected to analysis by GC-MS. Prior to analysis, pottery sherd samples were crushed and subjected to a chloroform/methanol extraction and derivatized. Sample preparation included precautions to eliminate and account for potential contamination from outside sources.

**Keywords:** Analysis, Art/Archaeology, Gas Chromatography, GC-MS

**Application Code:** Art/Archaeology

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Particle simulation methods that have been developed for separation science research can be broken down into two general classes: transport and molecular simulation.

Transport particle simulation methods utilize stochastic simulation of diffusion and may include convection and retention (1). These are utilized to solve transport problems where boundary conditions may be exceedingly complex and may involve simple or complex flow fields.

Particle simulation methods used in molecular simulation include the Configurational Bias Monte Carlo Method in the Gibbs Ensemble (CBMC/GE). CBMC/GE has been used to model solute-solvent-retentive interactions and accurately determine the mechanism and energetics of reversed-phase liquid chromatography (2-4).

Examples from the literature where transport simulation has been utilized to solve important practical problems in field-flow fractionation, capillary electrophoresis, chromatographic transport in packed beds and pore transport in chromatographic particles will be discussed.


Keywords: Computers, HPLC Columns, Liquid Chromatography, Separation Sciences
Application Code: General Interest
Methodology Code: Separation Sciences
Short columns of both superficially porous and totally porous sub-2 µm particles allow very fast separations, but practical limitations and other disadvantages of such small particles can be problematic. While sub-2 µm particles have demonstrated their utility in research studies, columns of larger particles are often better suited for most every-day applications. Superficially porous particles (SPP) in the 2.5 - 2.7 µm range provide nearly the same efficiency and resolution of sub-2 µm totally porous particles (TPP), but at one-half to one-third of the operating pressure. This performance advantage shown by these sub-3 µm SPP has led to the introduction of sub-2 µm SPP as a natural extension of this technology. However, the efficiency advantages of these very small particles often are not fully realized due to the difficulty of packing these very small particles into homogeneous beds. Additionally, the negative effects of extra-column volumes in most instruments cause peak broadening that is especially detrimental to the very low volume, narrow peaks that elute from high efficiency, small particle columns. The new 2.0 µm SPP described in this report provide most of the advantages and minimize most of the disadvantages of columns packed with sub-2 µm particles. The characteristics of these 2.0 µm SPP are described and studies comparing some present sub-2 µm SPP and TPP commercial columns for efficiency, column bed homogeneity and stability are presented. Several applications demonstrating the utility of 2.0 µm SPP will be shown.
Multidimensional Chromatography is a technique that keeps evolving. It is now well-recognized that the comprehensive version of two-dimensional chromatography can offer much larger peak capacity and has the ability to resolve complex samples where one-dimensional techniques lack the resolving power. This increased resolving power has been exploited for samples of both synthetic and biological origin.

In Multidimensional Chromatography, sample components are fractionated by two different column functionalities resulting in two different types of retention mechanisms. To achieve successful resolution of complex samples, dissimilar retention mechanisms are required to spread the separation into an area-based representation of zones.

The advantages of increased peak capacity, improved resolution and ability to handle complex samples in an efficient manner in our opinion far surpass the disadvantages of instrumentation complexity and time investment necessary to develop more than one separation procedure essentially at the same time.

This presentation will review the evolution of the technology, and will discuss the utilization of newer coupling approaches for the solution of complex problems related to samples of industrial and biological origin. Examples using gas and liquid phase separations will be presented, and the importance of the data obtained in the context of problem solving will be discussed.

Keywords: Chromatography, GC, HPLC
Application Code: General Interest
Methodology Code: Other
The characterization of conjugation sites in monoclonal antibodies (mAb) is critical in the early drug discovery phase since site-specific conjugation improves in-vivo stability and drug efficacy. The analysis of bioconjugates from in-vivo pharmacokinetic (PK) samples is useful for the determination of degradation and drug optimization for improved half-life. The complexity of identifying Lysine (Lys) conjugation sites lies in part to the large number of Lys residues present on the mAb scaffold. This has posed technical challenges to standard peptide mapping approaches. Therefore, an alternative strategy intended for a high throughput analysis has been investigated by coupling immuno-affinity capture coupled to reversed-phase liquid chromatography with mass spectrometry (2DLC/MS) detection. In this work, I will present methodology and examples of how 2DLC/MS was used to determine sites of conjugation for both an azetidinone (AZD) and N-hydroxysuccinimade (NHS) linker, as well as, the analysis of PK samples for determination of sites of degradation and optimization for improved half-life.
### Abstract Text

Liquid chromatography in all of its forms has proven to be an invaluable analytical technique over the past 3+ decades. As the separation challenges have increased over this time, practitioners and theoreticians alike have worked hard to develop and implement new approaches to meet these challenges. One major challenge is the separation of greater numbers of analytes in samples of ever-increasing complexity, and statistical considerations indicate that in most circumstances what is needed is a significant increase in peak capacity.

Comprehensive two-dimensional separations can provide a much higher peak capacity and greater probability of resolving the components in a given sample than conventional one-dimensional separations. However, the required instrumental setup is more complex and analysis times can be considerably longer.

Sequential-elution liquid chromatography (SE-LC) is a mobile-phase-centered approach to increasing the peak capacity and probability of a successful separation that only requires conventional HPLC instrumentation. In SE-LC, a sample comprised of different classes of compounds is introduced into a chromatographic system that is highly retentive for all compounds because of a weak mobile phase that is initially employed. The highly-retained compounds are then subjected to a sequence of mobile phases, each of which is designed to elute one class of compounds. The result of SE-LC is the sequential elution and separation of the sample components by class and also within class, i.e., each compound within a category is also separated from other compounds in the same category.

The theory of SE-LC is summarized and the results of numerous experiments utilizing different combinations of sequential, selective elution modes with several distinct columns or serial combinations of columns are reported for different samples containing various types of ionizable and neutral compounds. Pharmaceutical and other applications are discussed.

**Keywords:** Drugs, HPLC, Pharmaceutical, Separation Sciences

**Application Code:** Bioanalytical

**Methodology Code:** Liquid Chromatography
The concept of personalized medicine is predicated on an ability to comprehend a patient’s disease state in a highly informed manner that ideally illuminates an effective, molecularly-targeted treatment strategy. A growing body of evidence suggests that the simultaneous measurement of multiple unique biomolecular signatures from a single sample would be incredibly enabling in achieving such an informative diagnosis. Unfortunately, this is an analytical feat that currently not possible using established methods, thereby limiting the implementation of informative molecular diagnostic and theragnostic strategies in the clinical management and treatment of disease.

In response to these and other bioanalytical challenges that would benefit from high sensitivity, high level multiplexing capability, and scalable and cost effective sensor fabrication, our group has developed a biomolecular analysis platform based upon silicon photonic microring resonators. We have demonstrated the capabilities of this platform as applied to the detection of DNA, RNA, and protein biomarkers of disease, and have also shown its versatility in the facile screening of multiple types of biomolecular interactions. This talk will describe our efforts to develop this emerging platform in the context of creating multiplexed detection solutions for a range of challenges both in clinical diagnostics and fundamental biophysical interaction monitoring.

Keywords: Bioanalytical, Biosensors, Biotechnology
Application Code: Bioanalytical
Methodology Code: Sensors
We have recently obtained over 10,000 peptide identifications and over 2,000 protein identifications from a HeLa cell line using capillary zone electrophoresis in a single 100-minute analysis. These data are a 10-fold improvement in identifications using capillary zone electrophoresis over the previous state-of-the-art. We employed an electrokinetically-pumped nanoelectrospray interface to couple the electrophoresis capillary with an Orbitrap mass spectrometer. Based on these results, capillary zone electrophoresis is competitive with liquid chromatography for deep proteomic analysis.

Keywords:  Bioanalytical, Biological Samples, Capillary Electrophoresis, Proteomics
Application Code:  Bioanalytical
Methodology Code:  Capillary Electrophoresis
Microfluidics can be used to control multiple flows in single channels under conditions which prevent mixing of the flows. As we learn to direct and shape one fluid using another, an entirely new set of potential capabilities begins to emerge for optical components and sensors. Liquid waveguides can move light around corners and focusing cells in front of a laser beam can produce a microflow cytometer for point-of-care diagnostics or analysis of algae on unmanned underwater vehicles. Polymerizable streams can be used to fabricate fibers with predefined shape and highly consistent sizes over many meters in length. With sheath flow, we have aligned molecules within a subsequently polymerized matrix to create a fiber with optical anisotropy [1]. We have embedded metal nanoparticles in polymer fibers to control the color of the fibers and the plasmon resonance of the nanoparticles [2]. We can polymerize shaped fibers containing cells or decorated with recognition molecules to make filters that can simultaneously act as sensors [3]. Finally, we have demonstrated the integration of both bacteria and mammalian cells into hydrogel fibers [3,4]. Control of porosity and chemistry is essential to provide for cell viability. The bacterial can be used to detect environmental hazards such as arsenic or mercury, while the mammalian cells can be used to develop tissue-on-chip models, providing opportunity for imaging cellular interactions in three dimensions [5].


Keywords:  Bioanalytical, Biosensors, Integrated Sensor Systems, Lab-on-a-Chip/Microfluidics
Application Code:  Bioanalytical
Methodology Code:  Microfluidics/Lab-on-a-Chip
The multiplexed detection of nucleic acids and proteins via bioaffinity adsorption onto biopolymer microarrays has become a mainstream tool for biological researchers throughout the world, and Surface Plasmon Resonance Imaging (SPRI) is an established optical detection method for in situ multiplexed bioaffinity measurements that works very well and nanomolar concentrations or higher. In this talk, we describe the development of new SPRI methods that can detect and identify nucleic acids and proteins at picomolar and even femtomolar concentrations. These include (i) a near infrared SPR microscope for single nanoparticle-enhanced SPRI and digital bioaffinity adsorption measurements, and (ii) coupling SPRI with surface enzyme chemistries and on-chip templated biosynthesis the on-chip, and (iii) the application of single nanoparticle SPR microscopy to detect the uptake of bioactive peptides into single hydrogel drug delivery particles. These new SPRI ultrasensitive biosensing methods for detecting multiple DNA, RNA and proteins at extremely low (picomolar to femtomolar) concentrations will facilitate the identification of better biomarkers for early disease detection and strategies for post-treatment patient monitoring.
Protein separations represent a major technological challenge because of the multiplicity of forms that exist for even one
protein. Electrically driven separations, which are mainly electrophoretic sieving and isoelectric focusing, have been
widely used for decades, but they suffer from low throughput and are cumbersome in integrating with other separations
and with detection. Yet the simplicity of separating using a voltage rather than a pump should make these methods easy
to automate and integrate with mass spectrometry. What has been lacking is a medium that is easier to use than gels. We
have been exploring silica colloidal crystals as the replacement medium, giving faster separations with higher resolution
than gels, and in this work we discuss integration with detection methods as well as automation.

Keywords: Capillary Electrophoresis, High Throughput Chemical Analysis, Modified Silica, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Often new Mass Spectrometer instrument design begins development as a proof-of-concept or rapid prototypes in order to demonstrate a new advance in technology. An unnecessarily large fraction of such new designs under-perform when applied to field analysis due to factors unrelated to the new technology. Yet, end-users unfamiliar with the technology tend to view the technology itself as the failure. This talk will review some basic design concepts to consider at the onset of prototype development that can mitigate risks of reduced performance, or failure, in the field. Topics include reviews of design best practices for electrical, mechanical, fluid, and material system aspects. Additional topics will focus on human factors issues such as - ease of operation (especially by non-experts), ease of maintenance, and damage mitigation.
Recently, a backpack MS and a desktop Mini 12 MS systems have been developed and characterized for in-field chemical detection and point-of-care applications, respectively. In this presentation, a set of technologies recently developed will be introduced, which have been used to enable new capabilities or improve the functions of these miniature ion trap MS systems. A dual-LIT (linear ion trap) configuration has been implemented with the discontinuous atmospheric pressure interface (DAPI), which enables MS/MS analysis of multiple precursor ions with a single introduction of ions generated at atmospheric pressure. The scan speed of the miniature ion trap MS systems is thereby significantly improved when using ambient ionization source for analysis of non-volatile compounds. Prototype miniature pumps with new pumping technologies have also been tested for the miniature ion trap instruments. A 350g scroll pump developed by Creare provided adequate pumping for backing up the turbo pump, while operating extremely quite in comparison with the diaphragm pumps popularly used in the miniature MS systems. Development of sampling probes were also explored with several different designs, including the integration of a 130g turbo drag pump to a handheld unit along with a coaxial low temperature plasma source and a vacuum manifold containing a DAPI and a rectilinear ion trap. The synchronized discharge ionization method took advantage of the periodical pressure variations with the DAPI operation and enabled high sensitivity analysis of the volatile organic compounds in air. This method has been recently applied to detection of explosives and pesticides of low volatility. The recently developed slug flow microextraction nanoESI method has been shown to be powerful for direct analysis of biofluid samples. Its application to the analysis of drugs in urine and blood samples as well as the evaluation of the enzymatic functions will be introduced.

Keywords: Agricultural, Biomedical, Clinical/Toxicology, Food Safety
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
While the immediate concerns that come to mind regarding the concept of field-deployable MS instrumentation are the vacuum and power requirements, one must remember that there is no mass spectrum to be obtained without an ionization source that can vaporize/ionize the sample at hand. In the case of organic vapors, the energy-input required can be as simple as a filament or radioactive (beta particle) emitter. When one turns to sample forms wherein elemental/isotopic information is needed, the power requirements immediately go up as the sample must ultimately be degraded down to the atomic level; this is after whatever energy is required to affect the sampling and vaporization steps. The liquid sampling-atmospheric pressure glow discharge (LS-APGD) holds a unique position among the other atmospheric pressure glow discharges as it has demonstrated abilities in atomic emission, elemental mass spectrometry, molecular mass spectrometry, and ambient desorption mass spectrometry. These base capabilities are multiplied as the sample forms that are amenable to analysis include liquids, bulk solids, solution residues, and aerosols generated by laser ablation. This versatility is affected on a very small platform, with minimal supporting utilities. Specifically, the plasma is ignited between an electrolytic solution that is introduced at flow rates of 10-200 microliters/min at discharge currents of <60 mA, and d.c. powers of <50 W. The discharge volume is set by the electrode gap, but is usually on the order of 1 cubic millimeter. Importantly, ion sampling from the LS-APGD microplasma does not bring along the pumping overhead of high temperature/high gas flow plasmas such as ICP sources. It is believed that the LS-APGD microplasma holds a unique place among its peers and warrants investigation as a field-deployable ionization source for environmental and national security applications.

**Keywords:** Atomic Spectroscopy, Elemental Mass Spec, Mass Spectrometry

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Mass Spectrometry
Fieldable or portable analysis techniques are sought for in many environmental, and national security application areas. The development of new, robust approaches that provide smaller, simpler, cost-effective and analytical useful and defendable results remains a challenge however. This talk will survey these needs and provide concepts and ideas for unique, new approaches to fieldable MS that include development of new ionization mechanisms and sources as well as new MS separation technologies.

Keywords: Environmental, Isotope Ratio MS, Mass Spectrometry, Portable Instruments

Application Code: Environmental

Methodology Code: Mass Spectrometry
The oil and gas industry, and organizations that environmentally monitor deep ocean and coastal regions, have a need to detect and characterize light hydrocarbons in these regions. Hydrocarbons can enter the water column through natural seepage from the sea floor, or through unintended leaks and spills from drilling operations and transportation of oil and gas to shore. The ability to perform this analysis in situ greatly enhances achievable spatial and temporal densities. In addition, real-time, in-water chemical measurements allow for rapid decision-making, provide adaptive sampling strategies, and offer the possibility of tracking chemical gradients to trace leaking chemicals to their source. SRI International’s in situ membrane introduction mass spectrometry (MIMS) instruments can quantitatively detect light hydrocarbons in water at trace levels, while simultaneously monitoring the concentrations of all light, stable dissolved gases to a depth of 2000 meters.

We will discuss the design and operational parameters of our new in situ MIMS instrument, which is deployable on underwater platforms, such as the Bluefin BF-12 autonomous underwater vehicle (AUV). We will also present results from recent deployments of our MIMS systems to characterize hydrocarbon seeps and other features in the Gulf of Mexico, the Santa Barbara Channel, and Tampa Bay.

Keywords: Environmental/Water, Hydrocarbons, Mass Spectrometry, Portable Instruments
Application Code: Environmental
Methodology Code: Portable Instruments
Adverse Outcome Pathways (AOPs) in Human Systems Biology: In Vivo Discovery for Developing In Vitro Analytical Methods

The Air, Climate, and Energy (ACE) and Chemical Safety for Sustainability (CSS) programs at the U.S. Environmental Protection Agency (EPA) encompass broad-based research that includes assessment of the health and environmental impacts of anthropogenic and manufactured chemicals. One component of these programs is to develop efficient and accurate analytical methods for testing the relative toxicity and potential health effects of chemicals (and chemical mixtures) and to ultimately guide chemical prioritization for more detailed evaluation and regulation. Currently, high-throughput in vitro methods using specific targeted adverse outcome pathways (AOPs) are implemented at the cellular and molecular level with liquid phase micro-fluidics including liquid chromatography-tandem mass spectrometry (LC-MS/MS) analyses. New methodology is now under development to implement gas phase probe molecules for assessing specific metabolic pathways that have already been well defined in human or mammalian models and apply semi-real time detectors (optical or MS) to in vitro measurements. We propose two probe molecule and metabolite pairs that could be used for in vitro assessment of perturbations in specific liver enzyme systems: methyl-tertiary butyl ether (MTBE) metabolism to tertiary butyl alcohol (TBA) via CYP2A6 and sevoflurane metabolism to hexafluoroisopropanol via CYP2E1 in flow through-reactors using human cell lines. We expect to extend the gas-phase probe methodology to airway epithelial cells and analyze carbon monoxide (CO) production via heme oxygenase (HO) activity.
Adverse Outcome Pathways (AOPs) in Human Systems Biology: In Vivo Discovery for Developing In Vitro
Real-Time Mass Spectrometry for Rapid Throughput in Adverse Outcome Pathways Assessments

Chemical analytical procedures for in vitro analyses of biological media typically involve considerable time-consuming steps that limit sample throughput. Such constraints can be reduced by use of real-time mass spectrometry for both in vitro and in vivo analysis. The technique of proton-transfer-reaction mass spectrometry (PTR-MS) is a chemical ionisation based tool that allows for rapid and direct detection and quantitation of gas-phase volatile organic compounds (VOCs). Depending on the medium, this can be made either in vivo or in vitro. Probative analysis of exhaled breath gas for biomarkers, for example, can be made at a breath-by-breath frequency and deliver immediate chemical information that can be utilised for adverse outcome pathways (AOP) studies. On the other hand, in vitro analysis of other bodily media, e.g. in the form of the detection of VOCs in the headspace of fluids such as blood, urine or saliva, can offer rapid analytical throughput of samples for correlation with in vivo data to establish chemical pathways. This presentation will introduce the technology of PTR-MS, outline latest developments, and discuss potential applications for AOP assessments.

Keywords: Analysis, Biological Samples, Detection, Mass Spectrometry
Application Code: High-Throughput Chemical Analysis
Methodology Code: Mass Spectrometry
Abstract Text

Exhaled human breath consists of mixtures of molecules derived from the human exposome and from normal and abnormal physiologies. The advantages of breath over other bodily fluids are that it can be collected multiple times non-invasively from infants or adults without posing any risk to the patient or the person collecting the sample. Breath can be sampled or collected easily anywhere: in a physician’s office, in a clinic, in the field or in the home. Real-time monitors are currently being developed and these devices may be well-suited for field and epidemiological studies, particularly for studies in developing countries where collecting blood and urine samples are difficult without refrigeration.

If inexpensive portable real-time monitors can be developed for point-of-care testing then patients can be provided important information immediately and treatment initiated. Real-time breath analysis is ideally suited to personalized medicine, which is the future direction for clinical medicine. Moreover if devices are portable, simple to operate and relatively inexpensive then chronically sick patients could monitor their progress in their home and thereby minimizing their exposure to infections during routine visits to clinics.

Currently, breath analysis can be used to monitor disease progression, or monitor therapy. Breath analysis could be used for phase 1 and phase 2 clinical trials to monitor new drug therapy or to detect potential adverse effects. Since breath analysis is non-invasive and can be performed easily, it allows larger numbers of study subjects to be studied. Using larger numbers of study subjects, unusual adverse effects are more likely to be identified. This presentation will discuss the various analytical approaches that are being used to develop real-time breath monitors with particular emphasis to the use of mid-infrared QC lasers “Personalized medicine using mid-IR spectroscopy”.

Keywords: Biological Samples, Infrared and Raman, Membrane
Application Code: Biomedical
Methodology Code: Portable Instruments
Exposure to inorganic arsenic (iAs) early in life is associated with adverse health effects in infants, children, and adults, and yet the biological mechanisms that underlie these effects are understudied. The objective of this research was to examine the proteomic shifts associated with prenatal iAs exposure using cord blood samples isolated from 50 newborns from Gómez Palacio, Mexico. Levels of iAs in maternal drinking water (DW-iAs) and the sum of iAs and iAs metabolites in maternal urine (U-tAs) were determined. Cord blood samples representing varying iAs exposure levels during the prenatal period (DW-iAs ranging from <1 to 236 $\mu$g As/l) were analyzed for altered expression of proteins associated with U-tAs using a high throughput, antibody-based method. A total of 111 proteins were identified that had a significant association between protein level in newborn cord blood and maternal U-tAs. Many of these proteins are regulated by tumor necrosis factor and are enriched in functionality related to immune/inflammatory response and cellular development/proliferation. Interindividual differences in proteomic response were observed in which 30 newborns were "activators," displaying a positive relationship between protein expression and maternal U-tAs. For 20 "repressor" newborns, a negative relationship between protein expression level and maternal U-tAs was observed. The activator/repressor status was significantly associated with maternal U-tAs and head circumference in newborn males. These results may provide a critical groundwork for understanding the diverse health effects associated with prenatal arsenic exposure and highlight interindividual responses to arsenic that likely influence differential susceptibility to adverse health outcomes.

Keywords: Biological Samples, Environmental, Proteomics
Application Code: Biomedical
Methodology Code: Other
Abstract Text
While chronic disease and cancers are caused by both genetic and environmental factors, it has been estimated that 70-90% of disease risk is due to differences in environments. To investigate the role of the environment in disease etiologies, untargeted approaches are needed that can compare chemical profiles across groups in an unbiased fashion. Candidate biomarkers identified in such studies can then be targeted in population-based studies using simple blood collection methods, such as dried blood spot (DBS) sampling. While it is currently not possible to measure all chemicals in the human body in single experiments, important classes of reactive chemicals can be targeted. Here we report a novel approach using protein adduct (addition product) profiles as molecular fingerprints of environmental exposures, called “adductomics”. Because protein adducts persist in the blood for the life span of the protein, adducts represent an integration of exposures occurring over weeks to months (e.g., mean residence times are 28 days and 63 days for human serum albumin (HSA) and hemoglobin, respectively). Consequently, protein adducts serve as ideal biomarkers for measuring exposures to environmental chemicals when chronic exposure scenarios are of greatest interest. Once discordant adducts are identified in discovery-based experiments, we then apply targeted protein adduct methods to dried blood spots (DBS), which can be collected using a simple and minimally-invasive finger prick. To achieve unparalleled analytical sensitivity using extremely small volumes of blood, adducts will be quantified using a state-of-the-art Agilent 6490 QQQ mass spectrometer with a nano-chip HPLC interface. This analytical platform permits biomarker detection down to the attomolar concentration (10-15 mol/L) using micro-liter blood volumes that can be obtained from a single 3 mm punch of dried blood.

Keywords: Elemental Mass Spec, Environmental/Biological Samples, Mass Spectrometry, Peptides

Application Code: Environmental

Methodology Code: Mass Spectrometry
We show that mediator-tethered atomic force electrochemical microscopy (Mt/AFM-SECM), a local probe technique based on the direct contact between surface-confined redox-labeled molecules and an incoming microelectrode, complemented by redox-immunolabelling enables mapping the distribution of proteins on the shell of individual viruses. The perfectly ordered and known viral architecture allows single protein resolution to be demonstrated. This result makes Mt/AFM-SECM a powerful tool for viral nanotechnology as it allows both the topography but also the function (e.g. redox function) of functionalized viruses to be simultaneously characterized at the individual virus scale.
Most research on nanoparticle (NP) collisions with an ultramicroelectrode (UME) has been with “hard” NPs (e.g., metals, oxides, organics, and polymers). Recently, collisions of “soft” NPs, like emulsion droplets, biological cells, and viruses, have been described. The electrochemical response and the frequency of collisions allow one to extract information about the NPs and even study electrode reactions in fL and aL droplet reactors. An overview of this field will be given and recent advances described.

**Abstract Text**

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**Keywords:** Electrochemistry, Spectroelectrochemistry

**Application Code:** Nanotechnology

**Methodology Code:** Electrochemistry
## Abstract Text

Research on nanoparticle (NP) collisions at an ultramicroelectrode (UME) have focused on “hard” NPs (e.g., metals, oxides, organics, and polymers) and more recently on “soft” NPs (e.g., emulsion droplets, biological cells, and viruses). The electrochemical response and the frequency of collisions allows one to extract information about the NPs and can provide a useful approach to the study of electrocatalysis at single metal NPs and serve as the basis of sensitive electroanalytical methods. An overview of the rapidly developing field of metal NP collisions at a UME will be given and recent advances described.

### Keywords
- Electrochemistry
- Microelectrode
- Nanotechnology

### Application Code
- Other

### Methodology Code
- Electrochemistry
This talk will present our recent results in using single-molecule fluorescence microscopy to image photoelectrochemical reactions on single semiconductor nanostructures. We separately image hole and electron induced reactions, driven by light and electrochemical potential, and map the reactions at single reaction temporal resolution and nanometer spatial resolution. We also correlate the surface reactivity with the overall performance of each nanostructure in photoelectrochemical splitting of water.
In the first part of this lecture, I will provide some background material on the basic physical concepts underlying molecular plasmonics with an emphasis on SERS and LSPR spectroscopy. Next, I will turn to the area of single particle surface enhanced Raman spectroscopy (SPSERS). In this section I will provide some answers for several fundamental questions such as: (1) what is the largest possible enhancement factor (EF) and (2) what nanostructure produces the largest EF. Our approach to answering these questions involved the development of new tools using single nanoparticle SERS and single nanoparticle LSPR spectroscopy spatially correlated with high resolution transmission electron microscopy (HRTEM).

In the second part of this lecture, I will discuss very new results in single molecule SERS (SMSERS) and single molecule tip-enhanced Raman spectroscopy (SMTERS). These include: (1) the first SMSERS study involving nanofabricated surfaces rather than chemically synthesized nanoparticles; (2) a simultaneous isotopologue and bianalyte experiment; and (3) the study of isotopologues and fluctuation analysis in SMTERS.

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In the atomic spectrometry community, the use of plasma sources has become associated almost exclusively with elemental analysis. This trend is not surprising; after all, inductively coupled plasmas, glow discharges, and microwave-induced plasmas offer outstanding figures of merit for the determination of metals and metalloids. However, in other fields, these same sources, suitably modified, and others such as corona discharges can be used not only to ionize samples but also to desorb and fragment them. These alternative plasmas therefore offer promise for molecular and speciation analysis. In this presentation, the focus will be on plasma sources that are capable of molecular characterization and speciation. The first source is intended for the simultaneous emission-based examination of spots on a two-dimensional (planar) chromatographic plate, such as one from thin-layer chromatography or two-dimensional gel electrophoresis. Coupled with a monochromatic imaging spectrometer, the glow-discharge source enables the simultaneous detection and quantification of spots that hold metal-containing proteins or those stained with silver, gold nanoparticles, or metal-containing affinity tags used to identify functional groups. A second source is based on the flowing afterglow produced by an atmospheric-pressure glow discharge (APGD). Simple in design, construction, and operation, this source can ionize vapor-phase samples or can be used to volatilize solution or solid samples directly and to ionize the resulting molecules. The resulting mass spectra are amazingly simple, and consist mainly of the molecular ion or protonated molecular ion. Because of this spectral simplicity, simple mixtures of compounds can often be characterized without a prior separation. The plasma source is therefore useful for speciation analysis and for the burgeoning technique of ambient desorption-ionization mass spectrometry.

Keywords: Elemental Mass Spec, Mass Spectrometry, Molecular Spectroscopy, Plasma Emission (ICP/MIP/DCP/e
Application Code: General Interest
Methodology Code: Molecular Spectroscopy
Atomic Tools as Solutions to Molecular Problems

Laser Ablation Molecular Isotopic Spectrometry (LAMIS) – Direct Solid-Sample Isotopic Analysis Through All-Optical Means

Laser Induced Breakdown Spectroscopy (LIBS) is a convenient and powerful analytical tool for direct and fast elemental analysis at atmospheric pressure for samples of virtually all kinds. However, LIBS generally provides only elemental information of a given sample but not its isotopic constitutions. The development of the Laser Ablation Molecular Isotopic Spectrometry (LAMIS) technique allows isotope analysis by laser ablation.

The LAMIS technique measures the molecular spectra of those radicals that are produced when the ablated materials expand into the atmosphere. It is implemented similar to conventional LIBS elemental analysis, and can be measured simultaneously with LIBS under the same laser pulse. Because isotope shifts in molecular spectra (LAMIS) are orders of magnitude larger than those in atomic spectra (LIBS), it is possible to determine isotopes at atmospheric pressure by an all-optical means. Some previously published LAMIS works include isotopes from carbon, boron, hydrogen and strontium.

In this presentation, the theoretical principles of LAMIS will be overviewed, and the current status (e.g., analytical figures of merit as well as challenges) of this technique will be discussed. In addition to chemical analysis, LAMIS can also be used for fundamental studies in laser-induced plasma. For example, we recently employed the LAMIS technique to clarify the mechanisms leading to the formation of C[2][CN] molecules during laser ablation of an organic compound in ambient air, which also will be presented in this talk.

Keywords: Atomic Emission Spectroscopy, Atomic Spectroscopy, Elemental Analysis, Laser
Application Code: General Interest
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The recently introduced strategy for the analysis of rare earth-tagged antibodies in tissues using laser ablation-cy-TOF has gained a lot of attention due to the fast multielement imaging capabilities under atmospheric conditions. The method requires fast transport of laser-generated aerosols into the cy-TOF where quasi simultaneous detection of all isotopes of interest takes place. Furthermore, the spatial resolution for discrete sampling of the tissue has to be in the order of 1 micron to provide insights into the antibody distribution within single cells.

The research reported in the presentation will focus on various inhouse built laser ablation systems and ablation cell geometries suitable for fast screening of tissue are available and that the wavelength of the laser for tissues is less crucial as reported for geological samples. In addition, some attempts towards quantitative analysis will be discussed.

Keywords: Laser, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
**Abstract Text**

Glow discharge optical emission spectroscopy (GDOES) has been historically used for direct bulk analysis and depth profiling of solid samples. Recently, the capabilities of GDOES have been expanded such that laterally resolved information can be obtained from within the sample sputtered area. This is achieved by collecting the emitted light with a spectral imaging system and by operating the GD in pulsed mode and under high pressures. The potential impact of the new capability becomes apparent when one recognizes that traditional elemental mapping techniques are throughput limited such that large area samples require many hours for measuring an elemental landscape with sufficient pixel density. On the other hand, GDOES elemental mapping has shown it can yield chemical composition landscapes with several orders of magnitude faster acquisition times than traditional techniques. As a result, elemental mapping of large area samples has the possibility of being a tool for routine analysis and diagnostics, as opposed to being limited to fundamental studies. Currently, protocols are being developed for its use with biological thin sections which will open the development of imaging methods for molecules used for therapeutic compounds, contrasting agents, toxicology studies, and many more. Recent advances will be presented.

**Keywords:** Atomic Emission Spectroscopy, Atomic Spectroscopy, Bioanalytical, Imaging

**Application Code:** Bioanalytical

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
The road to a healthy, prosperous future for humankind and nature as a whole is dependent on scientific discovery to understand processes on a molecular level and to develop approaches to improve the symbiotic relationship between humans and nature. At the heart of these discoveries is the need for powerful analytical instrumentation to view natural processes of the past and to project a path of the future. One particular area of the analytical sciences, atomic spectroscopy, has been essential to understanding modern (<100 years ago) biological processes as well as that from the distant past (>100,000 years ago). Yet, this area is often labelled as a “mature field,” which implies there is nothing left to discover or no more problems to solve. As will be demonstrated here, there is still a need for better (i.e. faster, robust, portable, sensitive, etc.) tools to quickly provide a comprehensive assessment of species in a sample in situ.

This presentation will focus on a novel plasma source capable of providing molecular, structural, and atomic information of species when coupled with mass spectrometry. In this plasma-based ambient mass spectrometry experiment, the source directly removes molecules from a sample surface, ionizes the species, and transfers these ions into a mass spectrometer. The separation power and sensitivity of the mass spectrometer is used to resolve individual components in a complex mixture. By adjusting the operational mode of the plasma source, the produced mass spectra selectively consist of intact molecular ions, electron ionization (EI)-like fragments, or bare atomic ions. This plasma source can also be coupled with electrospray ionization to enhance atomic and molecular detection of large biomolecules. The plasma source design and operation will be presented as well as representative applications and mass spectra. Lastly, approaches to process and deconvolute complex mass spectra generated by this system will also be presented.
In 1970, essentially all infrared spectrometers that were sold to analytical chemistry laboratories were based on grating monochromators. By 1980, FT-IR spectrometers had made a large impact on the market and within a few more years, scanning grating spectrometers had become obsolete. After a brief introduction to the types of FT-IR spectrometers that were developed in the 1960s, I will describe the factors that gave rise to the capability to measure mid-infrared spectra rapidly and at reasonably high resolution. These factors included the report of the fast Fourier transform in the mid 1960s by Cooley and Tukey and the subsequent development of three items of hardware: minicomputers, small helium-neon lasers and the triglycine sulfate (TGS) pyroelectric bolometer. The first instrument to incorporate all these components was the Digilab FTS-14. Although each of these items has been replaced by higher-performance analogues, today’s FT-IR spectrometers bear a striking resemblance to the FTS-14, which can, I believe, be called the first FT-IR spectrometer of the modern era. In this talk, I will describe the development of this instrument in 1969 and 1970 and relate some of the events that occurred in this period.

Keywords: FTIR, Infrared and Raman, Molecular Spectroscopy, Spectrometer
Application Code: Other
Methodology Code: Vibrational Spectroscopy
Synthetic Rubber, Spectros and War: The Start of Beckman Instruments in IR

This talk will examine the development of IR instruments at Beckman Instruments at the behest of the US government and in support of the national synthetic rubber effort. The talk will treat how and what decisions were made to create an instrument that could provide standard readings that could be easily shared between the researchers on this major military effort.

Keywords: History of Chemistry, Infrared and Raman, Instrumentation, Spectrophotometry
Application Code: General Interest
Methodology Code: Other
The speaker is old enough to have known many of the pioneers of infrared spectroscopy personally. This talk will present anecdotes and pictures concerning them. Among the stories will be an account of Harrison Randall's large parabolic mirror, how the American Cyanamid Co.'s infrared laboratory acquired Russian rock salt, Richard C. Lords's near catastrophe, Bob McDonald's slit curvature problem, how Gerhard Herzberg learned about his Nobel Prize, and Lionel Bellany's purchase of a lady's house in Paris.

**Keywords:** History of Chemistry, Infrared and Raman, Molecular Spectroscopy, Vibrational Spectroscopy

**Application Code:** Other

**Methodology Code:** Vibrational Spectroscopy
This talk will cover the history of IR instruments at Perkin-Elmer starting in the 50's with the single beam Model 12, the breadboard of which came from the American Cyanamid Corporation. The talk will also cover the major developments that followed Model 12, i.e., the development of a) the double beam Model 21, b) the low cost 137, c) the introduction of gratings, digital data handling, Fourier Transform (FT) instruments, and low cost versions of FT. The influence of competitive pressures will be discussed. Throughout, the contributions of Abe Savitzky will be noted as appropriate.
### Session # 300  Abstract # 300-5

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#### Abstract Text

I trace the early development and marketing of the Baird Associate dual-beam infrared spectrometer. My story makes clear how many variables beyond instrumental accuracy, precision and reliability contribute to success or failure.

#### Keywords
- Instrumentation
- Method Development
- Molecular Spectroscopy

#### Application Code: Other

#### Methodology Code: Other
Cells must acquire significant quantities of metals ions for use in a multitude of essential processes. While fluxes of alkali and alkaline earth metal ions play well-established roles in controlling cellular physiology, recent studies have shown that dramatic time-dependent fluctuations in intracellular transition metal concentrations control key steps in development, proliferation, differentiation and growth. Major barriers to understanding these processes include the low sensitivity of methods for determining concentration changes in small volume elements and maintaining the integrity of subcellular metal distributions during sample preparation. Here we address these issues and correlate results from several different ultrasensitive methods for direct analysis of changes in metal concentrations in single cells and in subcellular compartments, some as small as 250 nm diameter vesicles. Quantitative single cell X-ray fluorescence microscopy (ACS Chem Biol, 2011,15;6(7):716) and tomography experiments (Nature Chemistry, in press) at the APS (Argonne National Laboratory) open the door to the discovery of new signaling roles for zinc fluxes. Even higher spatial resolution of zinc distributions is achieved using a Hitachi 2300 STEM microscope equipped with dual EDS detectors. Finally new fluorescent probe molecules deployed in live cell confocal fluorescence experiments complement these physical methods and reveal how fluxes of billions of zinc atoms in the time course of seconds to hours are used by the cell to control key physiological decisions at the earliest stages of mouse, cow and primate development, including oocyte maturation, fertilization and early embryonic development. Thus, zinc fluxes involving the movement of from millions to billions of metal ions between compartments of a single cell, or between the intracellular and extracellular environment are emerging as key cell cycle regulatory events in biology.

Funding: Keck Foundation, NIGMS and NICHD.
The detection and quantification of biological trace metals is of critical importance for a comprehensive understanding of metal homeostasis in cells, tissues, and whole organisms. To this end, we designed and optimized water-soluble fluorescent probes for sensing Cu(I) and Zn(II) within a biological environment. Systematic optimization of the ligand architecture as well as electronic tuning of the fluorescence reporter were key for realizing a Cu(I)-selective probe with a 180-fold fluorescence contrast and a limit of detection in the parts-per-trillion concentration range. To take advantage of two-photon excitation microscopy, which offers reduced phototoxicity, improved depth penetration, and negligible background fluorescence, we developed an emission ratiometric Zn(II)-selective fluorescent probe with optimized non-linear optical properties. The water-soluble probe retains a balanced two-photon cross section upon Zn(II) binding and responds with a strong shift of the emission band suitable for ratiometric imaging of labile zinc pools in live cells and tissues. As a complementary approach we employed synchrotron X-ray fluorescence (SXRF) microscopy and 3D microtomography to visualize and quantify the transition metal distribution in proliferating cells and in developing zebrafish embryos.
Fluorescent ion indicator is defined and the question of why fluorescent ion indicator is answered. The advantages of fluorescence over other methods and criteria for a good indicator is presented briefly. Development of fluorescent ion indicators is presented with examples of the most important ions: Calcium, Sodium, Potassium, Magnesium and proton(pH). The future of fluorescent ion indicators and new developments from Teflabs concludes the presentation.
Fluorescence Sensing of Biologically Important Metal Ions: Detection, Speciation, Quantitation and Loc

Sensing Lead through Small Molecule Fluorescence

Lead toxicity is one of the most common environmental problems, particularly in older cities. Complications due to lead exposure can affect virtually every soft tissue in the body. Thus, the ability to detect small amount of lead is of paramount importance. We have developed a new class of fluorescent molecules that can detect lead in solution. This molecule has a dithiolene-based binding site, and thus differs from other commonly used fluorophores with more hard donors such as oxygen or nitrogen. Our studies suggest that the molecule can serve as a sensitive and selective fluorescent sensor for lead in a wide pH range. In this presentation we discuss the details about the material, lead binding affinity, and the selectivity against other ions in aqueous solution.

Keywords: Fluorescence, Metals
Application Code: Environmental
Methodology Code: Fluorescence/Luminescence
Metal ions perform essential functions in all facets of life. Analysis of metal ion concentration and function in live cells and tissue is a topic of considerable importance in virtually every field of biology. Such analysis is made challenging by several factors, among which the following three are prominent: 1) large metal ion concentration variations in cyto and in vivo; 2) dynamism in metal ion concentration; 3) detection selectivity amongst metal ions can be very difficult using various means of analysis. Small molecule fluorescence-based sensors have been developed for many physiologically relevant metal ions, including Ca2+, Zn2+, Pb2+, Na+, and K+. Key to successful molecule and analysis design is achieving physiologically appropriate metal ion affinity, sufficient selectivity among potentially confounding metal ion species, live cell permeability of probes, and sufficient S/N ratios to accurately determine the metal ion concentration of interest in various experimental systems. Detection is commonly achieved via fluorescence spectroscopy in multiwell plates, fluorescence microscopy, and flow cytometry. The talk will provide several examples of fluorogenic sensors from the metal ion list above, deployed in various experimental systems ranging from high throughput screening to live tissue.

Keywords: Biological Samples, Biosensors, Fluorescence
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Understanding the role of nutrients and metabolism in diseases such as cancers requires functional omics, of which network metabolomics is a bedrock approach. Metabolites are often referred to as "nuts and bolts" of metabolism; the analogy is appropriate, in that each metabolite is used in many places for disparate purposes. Thus, despite excellent analytical signal-to-noise ratio (s/n) of modern analytical techniques, the information s/n of each metabolite is less than unity due to their participation in multiple pathways. However, stable isotope labeling can distinguish pathways and functions among otherwise chemically identical metabolites. Using stable isotope resolved metabolomics (SIRM), we have demonstrated the capability to elucidate key pathway/network metabolic reprogramming in cancers, in effect achieving functional proteomics via vivo enzyme assays, and unambiguously assigning up/down regulation of pathways for signaling/transcriptomic work. For example, glucose vs glutamine sources of carbon in cancers has revealed key aspects of resource allocation, including the up regulation of anapleurosis via pyruvate carboxylase. In fact, we initially elucidated this pathway in lung cancers resected from 13C glucose infused human subjects. This demonstrates the unique ability of SIRM for untargeted discovery of specific metabolic pathways directly in humans for maximal relevance, following up with identical experiments in animal and cell models for unprecedented congruence of results. Of course many technical challenges remain some which will be described, however the greatest challenges are a new horizon revealed by SIRM, that of complex metabolic pools corresponding to previously unknown dynamic compartmentation. This is a crucial area poorly addressed by all of the omics, in part due to lack of phenotypic information, which SIRM can help to remedy.

Keywords: Biomedical, Magnetic Resonance, Mass Spectrometry, Metabolomics, Metabonomics
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Isotope Innovations for Metabolomics-Mass Spectrometry

Use of Stable Isotopes and Metabolite Profiling for Functional Characterization of Dysregulated Gene Expression and Protein Function Seen in Diabetes Development

Advances in metabolomics, transcriptomics, proteomics and in vivo stable isotope based fluxomics have made it possible to interrogate metabolic pathways in unprecedented detail. The convergence of multi-omic approaches is especially attractive to scientists in the field of diabetes and metabolic disorders, as they attempt to identify metabolic control points for therapeutic intervention. We have developed a framework for the step wise interrogation of complex models of insulin resistance, using metabolomics and stable isotope based fluxomics as primary tools for examining the functionality of tissue specific changes in gene/protein expression, and protein post-translational modifications, seen in the evolution of the diabetic state. For example, our published work has shown fasted/fed changes in metabolic network lysine acetylation can be associated with substrate re-partitioning down metabolic pathways, affecting the development of the (pre-) diabetic state. Changes in mitochondrial fuel utilization and energetics may not only be affected by alterations in gene/protein expression and post-translational modifications, but also via lipidomic interactions with components of the OXPHOS chain. The understanding of the evolution of metabolic network dysfunction that results in a diabetic phenotype requires a systems approach dependent on metabolomics and fluxomics for integration of the spectrum of transcriptomic and proteomic responses seen.

Keywords: Bioinformatics, Data Mining, Gas Chromatography/Mass Spectrometry, Metabolomics
Application Code: Biomedical
Methodology Code: Gas Chromatography/Mass Spectrometry
Any toxic event ultimately represents a disruption of homeostasis, which may be viewed either at the level of the epigenome, the transcriptome, or the metabolome. In almost all cases the metabolome is the earliest responder. The route by which homeostasis is disrupted will depend initially on the mechanism of action of the toxin and converge into one or more irrecoverable (fatal) disruptions. Between the initial and fatal disruptions are a number of possible states. In this study we examine the metabolomic response of yeast to three well characterized toxins, Ketoconazole, Terbinafin, and 5-flourocytosine, as an initial attempt to see if the IROA protocol provides a reasonable approach to determining these states.
Traditionally, isotope-tracer studies have been performed with targeted analyses examining a limited number of known downstream metabolic intermediates. Here we describe an alternative workflow that leverages recent advances in mass spectrometry-based metabolomic technologies to track the fates of isotope tracers in a global, unbiased manner. To accomplish this isotope-based metabolomic workflow, we have developed both software and a database optimized to identify isotopically enriched metabolites. We have applied these technologies to study labeled glucose in cancer cells. While it is well established that cancer cells uptake increased amounts of glucose, the fate of this glucose has not been well characterized. We provide a comprehensive list of intracellular metabolites enriched from glucose and quantitate the fraction of consumed glucose that ends up in proteins, peptides, sugars, and lipids.

Keywords: Bioinformatics, Biological Samples, Mass Spectrometry, Metabolomics, Metabonomics

Application Code: Biomedical

Methodology Code: Mass Spectrometry
One of the most rapidly growing areas in analytical chemistry is metabolomics, much as it was for proteomics ten or fifteen years ago. One driver of that growth is the enormous investment of NIH common fund resources in metabolomics centers across the country. Global and targeted metabolomics are poised to have one of the biggest impacts on human health, and innovations in analytical chemistry will drive that impact.

One of the most important tools in metabolomics is stable isotope labeling, which is used in unraveling metabolic pathways, in measuring metabolomics flux, in calibration for quantitative targeted metabolomics, and most recently as a strategy for comparative global metabolomics studies.

This talk will focus on the utility of isotopic labeling in MALDI imaging mass spectrometry, particularly for metabolomic applications.

**Keywords:** Biomedical, Imaging, Laser Desorption, Mass Spectrometry

**Application Code:** Biomedical

**Methodology Code:** Mass Spectrometry
As a platform for analytical separations, microfluidic devices offer a number of potential advantages over conventional methods. Microfluidic separations have been described that demonstrate major improvements in speed, sample consumption, automation, integration, separation power, and ease of use. However, the vast majority of these methods have been limited by a lack of mass spectrometry detection. We have pioneered the development of a sensitive, stable, and efficient microchip electrospray interface that enables the integration of MS detection with microfluidic separation methods. These devices yield electrospray ionization (ESI) performance as good as any nanoESI emitters without sacrificing the quality of microfluidic separations. Most of our work has utilized microchip capillary electrophoresis (CE) with integrated ESI. Compared to CE-MS performed using fused silica capillaries, microchip CE-ESI-MS can achieve greater separation efficiency in shorter analysis times because the integrated injection and ESI functional elements greatly reduce extra-column band broadening. Microchip CE-ESI-MS could therefore be used simply to improve CE-MS for challenging applications such as the characterization of intact biopharmaceuticals and protein/drug conjugates, where achieving optimal separation efficiency is crucial for the success of the analysis. Additionally, this technology could be applied to new analytical methods, which take advantage of the high separation speed and integration enabled by the microfluidic platform. A number of examples will be presented including the analysis of biopharmaceuticals and proteolytic peptides.
Mass spectrometry (MS) is an efficient, sensitive, and highly specific detector for a wide range of compounds encountered in the analytical laboratory. Low-flow electrospray ionization, referred to as nanospray, has been the ionization method of choice to interface mass spectrometry to capillary separation techniques such as reverse phase LC, capillary electrophoresis, and related methods. Nanospray has enjoyed a great deal of success as the interface to both planar and capillary microfluidic systems.

LC and CE are inherently analog technologies. Considerable effort in method development and technical overhead (operator expertise) is required for success. High quality chemical separations take time, both to develop the method, as well as to achieve each individual separation per sample. Chemical separations in LC, for example, occur on the minutes to hours time scale. In contrast, MS is inherently digital. Ions are generated and analyzed at a rapid rate in discrete packets. MS is acquired on the microsecond to millisecond time scale. The resulting high economic cost of LC in LC-MS is limiting the adoption of MS in many applications.

Droplet based microfluidics is rapidly becoming a transformative technology for LC-MS. The segmentation of samples, standards, and mobile phase into 1- and 2-dimensional linear microfluidic arrays effectively digitizes LC. As a result the MS is no longer tied to the timescale of the separation. The mass spectrometer may now be used in entirely new ways.

Here we will present some of the fundamental advantages of nanospray for the coupling of high speed segmented droplet flow microfluidics. These unique characteristics preserve the integrity of the fluid flow, as well as the optimizing MS performance. A novel approach for in-line SPE using structured droplet arrays will be presented.

Keywords: Capillary LC, Liquid Chromatography, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry
Application Code: High-Throughput Chemical Analysis
Methodology Code: Liquid Chromatography/Mass Spectrometry
Investigations of metabolite profiles from endothelial cells cultured for multiple days on a hybrid polydimethylsioxane (PDMS)/glass chip will be discussed. To mimic the cardiovascular environment, aortic endothelial cells are cultured in a PDMS-based microfluidic platform with continuous flow imparting physiological shear stress (>15 dynes/cm²). Cells are viable and maintain physiological shape for longer than 7 days under these conditions. Novel cell lysing methods are employed to optimize reproducibility, reduce artifacts from sample preparation, and minimize manual sample preparation steps. Removal of enzymes and lipids from the metabolite lysate will be explored. Leveraging the porosity/low back pressures of monolith columns, flow based culture systems and sample preparation can be joined. Monolithic stationary phases will be used for metabolite preconcentration. Metabolites relevant to glucose metabolism and oxidative stress will be evaluated as a function of external glucose stimuli. Both MALDI and ESI-based mass spectrometry will be employed for metabolite identification.
Abstract Text

Mass spectrometry imaging techniques can currently obtain excellent spatial resolution for small molecules, but imaging of larger molecules is obtained at the expense of resolution. Novel approaches are needed to bring large biomolecule mass spectrometry analysis to the sub-micrometer size regime. Laser-based methods such as matrix-assisted laser desorption ionization (MALDI) using conventional laser focusing configurations are currently limited to a resolution of 10 to 100 µm by the ability to focus the laser using conventional optics. In this project, we are using an atomic force microscope (AFM) to image the sample and to focus the laser energy for sample ablation for analysis of the captured material by mass spectrometry. The system uses the AFM and tip enhanced laser ablation to transfer biomolecules from cell and tissue samples from the AFM stage to a microfluidic system for analysis by nanoelectrospray ionization or matrix-assisted laser desorption ionization mass spectrometry. The metal-coated tip acts as an antenna to focus the light in the optical near field to ablate a spot with a size of 100 nm to 1 µm. Initial studies indicate that amol quantities of peptides can be transferred and analyzed by mass spectrometry with high efficiency. We are investigating the use of separations and protein digestion directly on tissue samples and after sample transfer. Tip-enhanced laser ablation transfer provides a new method for high spatial resolution sampling, microfluidic processing, and mass spectrometry detection will enable the analysis minor biomolecule components of cells and tissue.

Keywords: Imaging, Laser Desorption, Mass Spectrometry, Microscopy
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Separations and mass spectrometry are critically important analytical techniques that are useful for a wide range of applications. But a limitation for both techniques is the requirement of laborious, multi-step sample preparation prior to analysis. This has led to great interest in the microfluidics community in the development of automated, integrated techniques to serve as a front-end complements to separation systems and mass spectrometers. Most of this work has relied on the conventional microfluidic device format of planar devices with enclosed microchannels, but a new format known as "digital microfluidics" has recently become a popular alternative. In digital microfluidics, droplets are manipulated electrostatically on open devices (with no channels) bearing an array of electrodes covered with a hydrophobic insulator. Digital microfluidics has significant advantages for sample preparation, including the capacity to handle both liquids and solids (with no risk of clogging), and the ability to address each droplet individually (allowing for absolute control over all reagents). In this talk, I will review a number of methods recently developed using digital microfluidics for processing samples upstream of mass spectrometry and separations, highlighting applications involving core needle biopsies, dried blood spot (DBS) samples, and solid phase microextraction (SPME) probes. I propose that these examples and others suggest that the combination of digital microfluidics with separations/mass spectrometry may be useful for a wide range of applications in laboratory science.
New Developments and Challenges in Laser Induced Breakdown Spectroscopy

Oceanic LIBS Spectroscopy: Concepts, Challenges and Tests in Mediterranean Waters

After decades of development in laboratories and land operations, chemical analysis of submerged objects is starting to become a reality. While the analysis of water at variable depth has been demonstrated in the past using fluorescence spectroscopy and Raman spectroscopy, determination of the atomic composition of submerged objects is much a more complex task. Technology based on laser-induced breakdown spectroscopy (LIBS) has been recently developed for such sub-sea operations. This paper will discuss the operating parameters of a marine LIBS analyzer. Metals, alloys, rocks, marble, concrete, can be analyzed at a depth of up to 50 m. The system has been tested in several coastal surveys in Mediterranean waters.

Keywords: Art/Archaeology, Atomic Emission Spectroscopy, Environmental/Soils, Laser
Application Code: Other
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The use of chemical analysis and comparison of materials that are of interest to forensic scientists is possible by the progression from fundamental research and method development, validation in several forensic laboratories and the publication of international standards. While analytical standards such as ASTM do not currently exist for the application of LIBS in forensic analysis, evidence is mounting that analytical LIBS is fit-for-purpose for the elemental analysis of materials including glass, paint, soils, precious metals, diamonds, paper and ink on paper. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has been called the “gold” standard for solid-sampling and high-sensitivity elemental characterization of materials providing sub-ppm detection limits while providing true quantitative analysis data that can be used in numerical/statistical hypothesis testing to determine “match” and also to populate databases that are useful to determine the probability of a match for a given elemental profile. More than 30 forensic laboratories around the world now routine employ the use of LA-ICP-MS for materials characterization but there is an opportunity for many more laboratories to adopt LIBS as an analytical alternative to the expensive and complex LA-ICP-MS methods. This presentation compares the analytical figures of merit of LIBS to LA-ICP-MS for the analysis of several matrices of importance to forensic scientists. A comparison of the type of information gathered from LIBS and how the data is interpreted is also presented. Recent advances in instrumentation and data analysis schemes now makes analytical LIBS a viable alternative to LA-ICP-MS and other elemental analysis methods within the forensic science laboratory.

**Keywords:** Atomic Spectroscopy, Elemental Analysis, Forensics

**Application Code:** Other

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
There is a well-known and urgent need in the fields of medicine, environmental health and safety, food-processing, and defense/security to develop new 21st Century technologies for the rapid and sensitive identification of bacterial pathogens. In only the last ten years, LIBS has become a potential candidate technology for this application. LIBS’ significant advantages over current as well as other competing emerging technologies include speed (< 1 sec analysis), portability, robustness, lack of consumables, little to no need for sample preparation, no need for genetic amplification, no need for user expertise, and the ability to identify all bacterial pathogens without bias (including spore-forms and “viable but non-culturable” specimens).

In this talk I will present the latest achievements of our lab to more fully develop LIBS-based bacterial sensing. We have uniquely identified species from over five bacterial genera with high-sensitivity and specificity (chart 1). We have shown that bacterial identifications are unaffected by environment, nutrition media, or state of growth. We have shown that accurate diagnoses can be made on autoclaved or UV-irradiated specimens (chart 2). Efficient discrimination of bacteria at the strain level has been demonstrated. A rapid urinary tract infection diagnosis has been simulated with no sample preparation (chart 3). We have shown that efficient discrimination is possible in contaminated or “mixed” samples (chart 4) and a variety of autonomous multivariate analysis algorithms have been investigated.

I will then review the challenges facing us as we transition from laboratory demonstration experiments to more commercial biolab techniques and instrumentation. These challenges include: identifying a suitable substrate for mounting the bacteria, concentrating a number of cells for sufficient signal-to-noise from a clinical specimen, and introducing a limited number of cells reproducibly into the analytic spark.

**Keywords:** Atomic Emission Spectroscopy, Biomedical, Biotechnology

**Application Code:** Biomedical

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
In this presentation, we review the various LIBS applications to combustion science including combustion products and flame diagnostics. The measurements of temperature, fuel concentration and equivalence ratio using LIBS in various flames have been investigated. Atomic emission from laser-induced plasma of various flame conditions was recorded. The intensity ratios of elemental lines of N, H and O present in the LIBS spectra were found correlated with the equivalence ratio. The technique has successfully tested with various flames (e.g. methane/air, biodiesel flames, kerosene/air mixture, internal combustion engines, etc.). LIBS is also capable of being used as an engine health monitor to detect trace amount of metals emerging from any part of a engine during a diagnostic test. By comparing LIBS spectra and standard atomic emission from a rocket engine simulator, it was found that the LIBS technique has several advantages over standard emission methods to serve as a rocket engine health monitor due to its ability to provide time and spatially resolved measurements and improved detection limits for the metallic species. The various experimental parameters that are important to combustion application will be addressed. Various data processing methods to improve measurement accuracy will be compared. The possible other combustion applications will also be discussed.

Keywords: Analysis, Atomic Emission Spectroscopy, Atomic Spectroscopy, Plasma
Application Code: High-Throughput Chemical Analysis
Methodology Code: Atomic Spectroscopy/Elemental Analysis
New Developments and Challenges in Laser Induced Breakdown Spectroscopy

Ultrafast LIBS for 3D Chemical Imaging

In this work we use femtosecond LIBS coupled with advanced visualization capabilities for the elemental imaging of Li-ion battery components including anodes, cathodes, solid electrolytes and interfacial layers. Two-dimensional (2D) layer-by-layer mapping, 2D cross-sectional imaging and three-dimensional (3D) volume rendering of major and minor elements are presented. The elemental distributions are correlated to the electrochemical performance and serve to identify strategies to improve next-generation high energy density Li ion technologies.

Keywords: Electrochemistry, Energy, Laser, Ultra Fast Spectroscopy
Application Code: Materials Science
Methodology Code: Atomic Spectroscopy/Elemental Analysis
New Developments in Doping Detection

Exploring the Potential of High-Throughput Solid-Phase Microextraction for Analysis of Prohibited Substances in Urine, Plasma, Blood and Saliva

Drug testing for sports doping control is a complex and very challenging task. Firstly, the List of Prohibited Substances, updated annually by World Anti-Doping Agency (WADA), includes numerous classes of banned compounds having different chemical and pharmacological properties. Secondly, monitoring of these substances by anti-doping testing laboratories needs to be fast and efficient to analyze a large number of samples. Special attention must be paid to the sample preparation steps prior to instrumental analysis. Thus the objective of current research was to develop an automated method for the simultaneous multi-residue analysis of doping substances from urine, plasma, blood and saliva using solid phase microextraction (SPME) followed by LC-MS(/MS) and GC-MS analysis.

In this talk we will present the first successfully developed and validated SPME protocol, which requires very little sample handling for simultaneous analysis of more than 100 prohibited doping substances from urine. The optimization of method will be demonstrated and the results of validation according to the Food and Drug Administration (FDA) and WADA criteria will be provided. Additionally, aiming to improve the coverage of most polar prohibited drugs, the use of hydrophilic-lipophilic balance (HLB) SPME coatings for direct extraction of such substances from human plasma will be shown. We will also discuss the potential of using saliva as an alternative to urine and blood testing. We will demonstrate the use of HLB thin films for the extraction of the compounds followed by solvent and thermal desorption and with subsequent LC-MS and GC-MS analysis, respectively. Full validation of the method including precision, accuracy and matrix effect will be presented.

Authors would like to acknowledge the WADA for financial support

Keywords: Biological Samples, Drugs, Method Development, SPME
Application Code: High-Throughput Chemical Analysis
Methodology Code: Sampling and Sample Preparation
Enantiomeric separations have played a pivotal role in pharmaceutical science and drug development for over 25 years. While some consider the technology to be mature, new advances and developments have continued to the present time in both analytical and preparative separations. Furthermore the “chiral separations” are providing valuable information in many other fields. Recently these techniques were used to show that “natural” substances in unregulated nutritional supplements were likely synthetic stimulants. Many neurotransmitters are chiral and can be indicative of altered vs. normal brain chemistry. New techniques developed in our laboratory have produced ultra-fast enantiomeric separations, often in less than 20 seconds. A comparison of these new vs older separation approaches will be made and discussed in terms of doing “real time” analysis of the drugs and/or their effects on biological systems.
Clandestine laboratories constantly produce new synthetic cannabinoids to circumvent legislative scheduling efforts, challenging and complicating toxicological analysis. New designer drugs identification is an important anti-doping issue; however, synthetic cannabinoids metabolism and appropriate urinary target analytes are frequently unknown. With more than a 100 new synthetic cannabinoids identified in the last 6 years, these novel psychoactive substances are the emerging face of drug abuse.

We determined the most important urinary metabolites of new synthetic cannabinoids for which no metabolism information was available, and developed and validated qualitative and quantitative LC-MS/MS assays. We first incubated the drugs with human liver microsomes to determine the drug’s metabolic stability. New synthetic cannabinoids were incubated with human hepatocytes, followed by high-resolution mass spectrometry (HRMS) characterization of the synthetic cannabinoid metabolic profile. To date, this approach determined the metabolic profiles of XLR-11, AKB-48, RCS-4, RCS-8, STS-135, UR-144, AB-PINACA and 5F-AB PINACA. These data enabled laboratories to search for these metabolites to identify drug intake, tie adverse effects to specific synthetic cannabinoids, and educate the public on the danger of these new drugs. In addition, reference standard manufacturers could identify the most appropriate metabolites to synthesize and market.

Our latest HRMS urine method simultaneously identifies the most current 47 synthetic cannabinoid metabolites from 21 synthetic cannabinoid families. SWATH™ MS data were acquired in positive electrospray mode. Identification criteria included molecular ion mass error, isotopic profiles, retention time and library fit criteria. This non-targeted acquisition technique enables easy addition of new synthetic cannabinoid markers and the ability to return to the data later to search for newly identified novel psychoactive substances.
On-site sample preparation is an analytical approach based on direct sampling from the system under investigation. It has the advantage of combining sampling and sample preparation in to a single step, thus generally is fast, minimizes the potential sources of error and eliminates the risks for analytes instability. For such analysis solid phase microextraction in thin film geometry (TF-SPME) can provide robust and convenient in vivo sampling offering in the same time faster analysis and higher extraction recovery (i.e. better sensitivity) due to large surface to volume ratio.

In this study, TF-SPME in coated blade and membrane formats were used for in vivo and ex vivo saliva analysis by comparing the LC and GC based coverage of analytes with a single type of extraction phase. Due to applicability for wide range of polarity of analytes as well as thermal and solvent stability during the desorption, hydrophilic lipophilic balanced particles (HLB) were chosen as extraction phase and used for fast (5 min) in vivo and ex vivo sampling. The results of metabolomic profiling of the saliva are indicating that even 5 min in vivo sampling using TF-SPME followed by GC and LC analyses provides complementary coverage of wide range of analytes with different physical and chemical properties. To demonstrate the applicability of the method for doping analyses, the method was validated successfully for simultaneous quantification of 49 prohibited substances with limit of quantification (LOQ) ranging between 0.004-0.98 ng/mL. Moreover, the method was also validated and successfully applied for determination of endogenous steroids in saliva where the concentrations of the analytes are substantially low. The developed assay offers fast and reliable multiresidue analysis of saliva as an attractive alternative to the standard analysis methods.
We report here the first use of oligonucleotide-modified substrates as diagnostic tools for the direct identification of mutations in the influenza virus genome related to virulence. In these studies we target: 1) the N66S gene mutation within the PB1-F2 protein, and 2) neuraminidase (NA) stalk deletion mutants. The method employed 5’-thiol-modified ssDNA sequences as probes to capture RNA isolated from avian and reverse genetics influenza viruses containing low virulence or high virulence determinants. We used a label-free and amplification-free optical read-out method, i.e. Raman spectroscopy, to determine the efficacy of binding. The Raman spectra of both high virulence and low virulence DNA-RNA target complexes showed high similarity; therefore, multivariate analysis was used to identify target binding. Binary classification models were developed to distinguish complementary from non-complementary DNA-RNA target hybrids. The SVM-DA model that was developed using a radial basis function kernel resulted in calculated values of 95 – 100% sensitivity, specificity, and correct classification of the test samples with small root-mean-square errors of prediction.

These studies establish that optical-based Raman diagnostic methods are able to sensitively and accurately detect influenza virus RNA mutations linked to pathogenicity in emerging highly pathogenic avian and pandemic influenza viruses without amplification or labeling. The results are also the first demonstration of the use of real influenza viral RNA for direct identification of diagnostic indicators of influenza virulence. Future work will address the applicability and robustness of this platform for more relevant containing the target viral RNA in complex influenza isolates.

Keywords: Bioanalytical, Biomedical, Chemometrics, Surface Enhanced Raman
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
## Session Title
SERS for 'Oomics

## Abstract Title
Perm-Selective SERS Substrates for Direct Drug Metabolite Detection

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### Abstract Text
Directly detecting low concentrations of small molecules relevant in genomics, metabolomics, proteomics, etc. is often limited by similar molecular structures and function of the target species as well as complex sample matrices. When nanoparticles are used to facilitate detection, an addition limitation of signal irreproducibility from changing surface functionality further complicates detection. Often, biological recognition elements in these sensors are composed of antibodies, functional groups, nucleic acids, etc. Because surface recognition layers can exhibit variability as a function of temperature, matrix, shelf life, and pH; surface chemistry and quality control measures that promote both nanomaterial stability and responsiveness are vital and motivate our investigations. In this presentation, the design of stable, bio-responsive plasmonic nanostructures for the detection of biologically-relevant metabolites in a high throughput, sensitive, and selective manner thereby combating current dynamic metabolite detection limitations. Localized surface plasmon resonance (LSPR) spectroscopy and surface enhanced Raman scattering (SERS) will be discussed for applications in the direct, qualitative and quantitative detection of small biologically relevant molecules. In the future, exploitation of these perm-selective surface chemistries on SERS substrates for the direct transport and high throughput and sensitive detection of trace metabolites in complex sample matrices is expected to provide new opportunities for metabolomic analyses.

### Keywords:
- Materials Characterization
- Nanotechnology
- Raman
- Sensors

### Application Code:
- Nanotechnology

### Methodology Code:
- Sensors
Surface-enhanced Raman spectroscopy (SERS) is particularly powerful for biosensing because it can obtain the fingerprint information of the system we are interested in under the aqueous and ambient conditions, but with a much higher sensitivity compared with normal Raman spectroscopy. To obtain SERS signal of biomolecules (including amino acids, proteins, metabolites, and etc.) with very good reproducibility and high sensitivity is extremely important for a wider application of SERS in biological systems and is also a great challenge for SERS.

To address the issue, we proposed two direct detection methods. One is to mix the target molecules with metal colloids of high concentration and the detection is performed during the drying process, and the maximum sensitivity can be achieved right before the complete drying of the colloidal solution. This technique is called metastable SERS technique, which allows the SERS detection of 20 common amino acids and some important peptides with a highly reproducible signal.

The second method is to modify the surface of SERS-active nanoparticles or solid SERS substrates with some halide ions. Proteins were found to interact with the modified substrate via electrostatic interaction. The SERS signal of protein is enhanced by at least 1000 time over the solution species, but showing almost identical feature to that of the solution signal of proteins. This method allows the quantitative analysis of proteins and mixture of proteins. We further successfully extended this method for the DNA detection.

**Keywords**: Biospectroscopy, Nucleic Acids, Protein, Surface Enhanced Raman

**Application Code**: Genomics, Proteomics and Other 'Omics

**Methodology Code**: Vibrational Spectroscopy
The ability to identify and quantify multiple chemical analytes in biological fluids is key to utilizing the diverse molecular composition for biomedical diagnostics. In particular the array of metabolites found in biological fluids can provide a larger picture about a person’s health. The current state of the art is a separation followed by mass spectrometry, which can be challenged when identifying many relevant small molecules. Our laboratory has developed a high throughput surface enhanced Raman (SERS) sensor that provides complementary detection to mass spectrometry and improved limits of detection appropriate for molecules in biological fluids. Preliminary results indicate utility for both targeted metabolomics, but also the potential for metabolic profiling. In this presentation we will discuss the development of the flow detector, results from coupling the detector to chemical separations, and the application of this sensor to the detection and quantification of metabolites in biological fluids.
Abstract Text
Dried blood spot (DBS) sampling, as a microsampling technology, reemerged and has attracted great interest in the pharmaceutical industry as a viable alternative to plasma/serum sample collection for toxicokinetic (TK) and pharmacokinetic (PK) studies. One of the main challenges to a wide application of this technology is the sometimes negative impact on the assay performance by study sample hematocrit. One solution to address this issue is to implement accurate sample volume collection for each DBS sample and perform the sample analysis using the entire DBS sample. Depending on the type of study, implementing this accurate pipetting can be challenging. The ideal characteristics for dried matrix microsampling technology should include the ease of use (during sample collection and sample analysis), consistent and accurate sampling volume, etc. BMS, along with other industry peers, has been involved in the evaluation of a new microsampling technology called Volumetric Absorptive Microsampling (VAMS™). This presentation will discuss the preliminary evaluation results and potential advantages /further improvement need of using this technology.

Keywords: Bioanalytical, Biological Samples, Sampling, Tandem Mass Spec
Application Code: Pharmaceutical
Methodology Code: Process Analytical Techniques
Extensive investigation of dried blood spot (DBS) microsampling over the last few years has stimulated a renewed interest in all forms of microsampling. In addition to the advantages of small sample volumes, dry microsampling formats may also offer significant logistical advantages for sample handling, storage and shipment. However, widespread adoption of DBS has been limited by concerns about regulatory acceptance and potential technical issues, notably the potential for hematocrit changes to affect method accuracy. These concerns have led to investigation of various wet microsampling techniques as well as the development of improved dry sampling devices.

None of the above concerns are truly significant in the context of supporting early drug discovery studies, where DBS can provide a simple and practical method for collection of very low volume blood samples. Such low sample volumes can provide unique opportunities for pharmacokinetic sampling in various pharmacological, pharmacokinetic and toxicological studies conducted in small animals, both reducing animal use and improving data quality. A number of examples will be presented where minimal volume DBS sampling has allowed PK samples to be collected in sensitive pharmacology or toxicology models, and for full PK profiles to be collected from individual animals in studies in mice.

More recently a novel dried blood sampling device has become available that reliably absorbs 10 microliter volumes of blood and does not appear to be affected by changes in hematocrit. Investigation of this device for use in mouse PK studies will be described including a detailed comparison with results obtained with (wet) whole blood sampling. In addition, validation of an ELISA method for a monoclonal antibody drug using this device will also be reported, where similar performance was demonstrated to previous validations conducted with serum samples and DBS cards.

Keywords: Bioanalytical, Biological Samples, Drug Discovery, Liquid Chromatography/Mass Spectroscopy
Application Code: Pharmaceutical
Methodology Code: Sampling and Sample Preparation
A Survey of Current Microsampling Techniques in Developing Analytical Methods from Whole Blood

A Novel Microsampling Device that Absorbs a Fixed Amount of Fluid for the Collection of Biological Samples to be Dried and Stored for the Later Extraction of Analytes

As a micro-sampling tools DBS (Dried Blood Spot) Cards are used because of their simplicity, the small volumes of blood collected, low cost, and ease of transport. In a normal work routine a small disk is punched out of the DBS card. The amount of blood in these sub punches varies because the hematocrit of a blood sample mediates its viscosity and, therefore, how far the blood spreads out on a card. This leads to a volumetric assay bias that is a function of sample hematocrit.

We will describe a device comprised of a porous hydrophilic "tip" that is designed to have a controlled internal porous volume of 10µL. When this tip is touched to any aqueous fluid it will rapidly wick the fluid into its internal volume through capillary action. We will describe a series of evaluations that show the use of this device as a volumetric blood collection tool that can be dried, and then extracted for analysis (like a DBS card), but that overcomes the volumetric assay bias due to hematocrit. We will present experiments illustrating the volumetric accuracy of the device (regardless of hematocrit), the wicking speed of the device as a function of temperature and hematocrit, and the rapid drying of the sample on the device for storage. Extraction methodologies and analysis of analytes from the tip are shown, along with example assays with hematocrit bias < ±15%, and studies of on-tip analyte stability over 14 days demonstrate less than ±20% overall assay bias.

Keywords: Biological Samples, Drug Discovery, Mass Spectrometry, Sample Preparation
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Advances in Clinical Infrared and Raman Spectroscopy

A Pilot Study for Malaria Diagnosis in Thailand Using ATR-FTIR Spectroscopy

New technologies to diagnose malaria must have high sensitivity and specificity to enable the detection of premature parasitic forms namely ring and gametocytes in peripheral blood. Diagnostic techniques must also be portable and robust to be transportable to remote communities on motorcycles. During the course of its life the malaria parasite transgresses through several developmental stages including a sexual and an asexual reproductive pathway. The intraerythrocytic asexual phase of the life cycle commences when merozoites invade red blood cells (Figure 1). The merozoites grow and divide in the food vacuole and progress through three distinct morphological phases known as the ring, trophozoite and schizont stages. The schizonts burst releasing the merozoites and the intraerythrocytic cycle continues. Instead of replicating, some merozoites in the RBCs develop into sexual forms of the parasite, called male and female gametocytes, which like the ring forms circulate in the bloodstream. The detection of the rings and gametocytes at low parasitemia in peripheral blood is critical for early diagnosis and treatment. We demonstrate that ATR-FTIR spectroscopy in combination with a partial least squares regression models has the required sensitivity and ease of sample preparation to become a standard for malaria detection and most importantly quantification. The project is now set for a clinical trial in Thailand and the results of that trial will be presented at the meeting.

Biomedical, Biospectroscopy, Detection, Medical

Biomedical

Biospectroscopy
Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in women worldwide. The development of disruptive technologies for diagnosis and prevention has, thus, become of prime importance to mitigate and potentially eliminate the impact of metastatic breast cancer-related morbidity and mortality. To this end, we seek to develop Raman spectroscopic assays for sensitive assessment of change in tumor burden to allow early detection of recurrence and to aid in the evaluation of new therapies while the tumor burden is low with the hope of improving cancer outcomes. In this context, the need of addressing both the field enhancement and the extinction of nanoparticle suspensions, however, has been underappreciated despite its substantive impact on the sensing performance. Here, we perform the systematic experimental investigation of SERS enhancement and attenuation in suspensions of gold nanostars, which exhibit markedly different behaviors in relation to conventional nanoparticles. We elucidate a remarkable relationship between the SERS enhancement and the localized surface plasmon resonance band, and probe the effect of the gold nanostar concentration on the signal propagation.

Keywords: Biomedical, Biospectroscopy, Infrared and Raman, Nanotechnology
Application Code: Biomedical
Methodology Code: Biospectroscopy
Kidney transplantation is the main treatment for end-stage renal disease, however close monitoring of post-transplant biopsies is required to monitor and identify subclinical complications. In this study, we focused on identifying biochemical markers associated with recurrent diabetic nepthropathy using Fourier Transform Infrared (FT-IR) spectroscopic imaging. FT-IR imaging is an emerging approach to obtain label-free images of the biochemical composition of tissue biopsies. Initial studies focused on identifying archival FFPE tissue blocks of 8 patients with no evidence of diabetic nephropathy and 8 patients with advanced diabetic nephropathy. Serial sections were acquired and stained with PAS or placed on an IR compatible substrate. High definition IR images were acquired. A diagnosis was made of the degree of diabetic glomerulopathy based on the PAS stain. IR spectra were extracted from the different glomerular and tubular structures and compared to identify biomarkers associated with diabetic nephropathy progression. A second study identified three transplant patients with multiple surveillance biopsies who underwent very rapid recurrent diabetic nephropathy within two years and three normal patients with no evidence of diabetic nephropathy after two years. A number of biomarkers were identified that were shown to change in glomerular and tubular structures associated with increasing severity of nephropathy due to diabetes. Increased levels of glycation and of the symmetric phosphate band were consistently found to be increasing in the cohort of patients that had multiple surveillance biopsies and underwent rapid diabetic nephropathy recurrence suggesting that these features may be used to be predictive of early changes associated with diabetic nephropathy. FT-IR imaging is potentially powerful adjunct to current pathological methods allowing for the label-free and rapid identification of early markers of transplant complications.

Keywords: Biological Samples, Biospectroscopy, FTIR, Microspectroscopy

Application Code: Biomedical
Methodology Code: Vibrational Spectroscopy
Advances in Clinical Infrared and Raman Spectroscopy

Neurosurgical Guidance Through Stimulated Raman Scattering Microscopy

Achieving maximal, safe resection is the central goal of brain tumor surgery. One of the greatest barriers to optimal surgical outcomes in brain tumor surgery is the ability of surgeons to accurately differentiate normal from tumor-infiltrated brain. We have applied a nondestructive, label-free technique, stimulated Raman scattering (SRS) microscopy to detect tumor infiltration within the human brain. Here, we used SRS microscopy to image specimens from series of neurosurgical cases. The resulting data suggest that SRS microscopy is a highly accurate method for detecting tumor infiltration within the human brain that holds promise for improving the surgical care and study of human brain tumors.

Keywords: Biomedical, Molecular Spectroscopy, Raman
Application Code: Biomedical
Methodology Code: Vibrational Spectroscopy
Advances in Clinical Infrared and Raman Spectroscopy
Multiplexed and Quantitative Bioanalysis Using SERS

Surface enhanced Raman scattering (SERS) is an analytical technique with several advantages over competitive techniques in terms of improved sensitivity and multiplexing. We have made great progress in the development of SERRS as a quantitative analytical method, in particular for the detection of DNA. However, the lack of quantitative data relating to real examples has prevented more widespread adoption of the technique. Detection of specific DNA sequences is central to modern molecular biology and also to molecular diagnostics where identification of a particular disease is based on nucleic acid identification. Here we demonstrate the development of new molecular diagnostic assays based upon SERS which have been used successfully for the detection of bacterial pathogens associated with meningitis using modified SERS active probes. The probes have been designed to give a specific SERS response resulting in discernable differences in the SERS which can be correlated to a specific DNA hybridisation event. In this presentation the simultaneous detection and quantitation of 3 pathogens will be demonstrated.

Functionalized nanoparticles are a key component of the modern bionanotechnology area. We favour the use of functionalised nanoparticles as sensing materials for the detection of DNA at ultra low levels, proteins and more recently as substrates for imaging approaches. We achieve this by combining functionalised silver or gold nanoparticles with the spectroscopic technique of surface enhanced resonance Raman scattering (SERRS). This combination can result in extremely sensitive and selective biosensing with unprecedented multiplexing capabilities. This presentation covers the full range of design, the optical properties and finally the biological properties of functionalized nanoparticles in relation to their assembly and how that relates to the provision of new biological knowledge.

Keywords: Bioanalytical, Biomedical, Biosensors, Surface Enhanced Raman
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Recently, a number of proof-of-concept studies have shown that molecular spectroscopic techniques such as infrared absorption and Raman are capable of distinguishing diseased from non-diseased cells and tissue based upon the inherent chemistry contained within the cells. The presentation will review recent advances in the development of Infrared absorption and Raman spectroscopic modalities for applications in clinical diagnostics. It, and the subsequent discussion, will be guided by the UK EPSRC Network, Clinical Infrared and Raman Spectroscopy for Medical Diagnosis (CLIRSPEC- http://clirspec.org/), whose aims are to move forward the clinical application of IR and Raman spectroscopy by identifying barriers to their implementation in the clinical environment and, through multidisciplinary sandpits, propose ways to overcome these barriers. Specific objectives include:

1. To develop our understanding of the interaction of light with clinical samples
2. To develop internationally recognised protocols for the preparation of cells, tissue and biofluids for clinical spectroscopy
3. To provide evidence of the power of spectroscopy for use in the clinical arena
4. To determine the requirements of instrumentation suitable for use in the clinic
5. To develop strategies for pre-processing and statistical analysis in clinical spectroscopy
6. To develop inter-group data sharing protocols and portal

The presentation will thus identify key considerations under each objective, laying the platform for the subsequent open discussion forum.

Keywords: Biospectroscopy, Chemometrics, Infrared and Raman, Sample & Data Management
Application Code: Biomedical
Methodology Code: Biospectroscopy
Electrochemistry at Nanoscale Structures

Electron Propagation Through Redox-Active Microdomains in Thin Films of Side-Chain Ferrocene-Containing Diblock Copolymers

Thin films of redox-active block copolymers can be used as mediators for electrochemical enzyme sensors and as platforms for redox-controlled molecular deposition. For these applications, it is important to understand how to control the electron propagation efficiency of nanoscale redox-active microdomains formed in these films. This presentation will report our systematic studies on electron propagation through ferrocene-containing microdomains with different nanoscale morphologies. Specifically, three polystyrene-poly(2-(acryloyloxy)ethyl ferrocenecarboxylate) diblock copolymers (PS-b-PAEFc) with different PAEFc volume fractions (0.17, 0.30, 0.47) were synthesized by two-step atomic transfer radical polymerization. Their thin films were prepared on gold substrates via spin-coating, and characterized using spectroscopic ellipsometry and atomic force microscopy. Their electrochemical properties including the percentages of reactive ferrocene moieties and electron propagation efficiency (apparent diffusion coefficients) were quantitatively assessed using cyclic voltammetry and chronocoulometry.

Keywords: Chemically Modified Electrodes, Electrochemistry, Nanotechnology, Polymers & Plastics

Application Code: Nanotechnology

Methodology Code: Electrochemistry
An important semiconductor nanocluster (SCNC) property is the propensity to accept and donate electrons, making them ideal candidates for use in solid-state devices. In order to maximize charge generation and transport in these devices, ligand-coated SCNCs must be considered as single, charge-state definable, inorganic-organic hybrid molecules (hard inorganic core and soft organic surface), where fundamentally new electronic, photophysical, and electrochemical properties emerge at the SCNC-ligand interface. We have investigated the mechanism of electronic interaction between strongly quantum confined CdSe SCNCs (<2.0 nm diameter) and surface passivating ligands through combined optical and electrochemical techniques. Solution phase electrochemical measurement showed that the highest occupied molecular orbital (HOMO) peak position and columbic interaction energy of electron-hole pairs of ligand-coated SCNCs were strongly influenced by the chemical functionality of the surface passivating ligands. Quantification of HOMO position and interaction energy will provide insight into the mechanisms controlling the interfacial electronic interaction between SCNCs and surface ligands, which together improve the overall stability of hybrid molecules and control the generation and transfer of charge carriers.

**Keywords:** Electrochemistry, Energy, Materials Science, Nanotechnology

**Application Code:** Fuels, Energy and Petrochemical

**Methodology Code:** Electrochemistry
Our group develops electrochemical methods to study semiconductors in colloidal suspension. In one example, we study photocatalytic processes of colloidal semiconducting nanoparticles. We have demonstrated the detection of individual anatase nanoparticles interacting with an electrode by photoelectrochemical currents. These interactions carry information about the photochemical process at the single nanoparticle level of the electron transfer reactions for the photooxidation of methanol, proposed as a model system. We first demonstrated the detection of titanium dioxide nanoparticles where the step-wise increase in current was assigned to the particle by particle contribution of the photocurrent (J. Am. Chem. Soc., 2013, 135, pp. 10894–10897). The step by step detection corresponds to the so-called sticking interactions where the irreversible attaches to the electrode surface but we have also been able to observe the transient interactions of the NPs with a microelectrode. The effect of NP aggregation as studied by dynamic light scattering will be discussed in this seminar. Also the effect of using different electrode materials on the photocurrent will be covered. Our more recent advances include detecting nanoparticles that have been modified with a dye with used for the dye-sensitized solar cells. We have also been able to detect the NP interactions of different materials, such as CdSe bare and covered with a layer of ZnS.

Another example covers studies of size-dependent electrochemical properties of ZnO nanoparticles. The NPs are reduced using a Hg microelectrode to reduce the Zn ions in the ZnO lattice to a Zn(Hg) amalgam. We demonstrate the size dependence of the reduction potential of ZnO nanoparticles.
In this talk, I will present our research on single-nanoparticle electrochemistry. A carbon fiber ultramicroelectrode (UME) is used to study electrochemical and electrocatalytic activity of single gold/platinum nanoparticles. In this work, we use fast-scan cyclic voltammetry at ~500 V/s to measure transient voltammetric response of single nanoparticles undergoing free diffusion and collision with the probe UME. Kinetic information has been obtained from individual particles by analyzing their voltammetric response. This research demonstrates a new method to obtain chemically resolved information about transient particle–electrode interactions which is otherwise difficult to obtain with previous constant-potential techniques. Voltammetric responses can be obtained at single colliding NPs with millisecond time resolution prior to and after particle collision and immobilization. Such information can be particularly valuable for future mechanistic studies for electrocatalytic NPs such as particle–electrode interaction, particle activation and deactivation, and heterogeneous electron-transfer kinetics.

Keywords: Electrodes, Microelectrode, Sensors, Voltammetry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Electrochemistry at Nanoscale Structures

Selective Deposition of 1-2 nm Diameter Au Nanoparticles onto Electrode Surfaces by Migration

There has been an enormous effort to study the electrochemistry of metal nanostructures to gain a better fundamental understanding of nanoelectrodes, study single particle electrochemical reactivity, and exploit nanostructures for electrocatalysis and electrochemical sensing. Our group has recently studied the electrochemical oxidation of metal nanoparticles as a function of size due to its relevance to nanoparticle stability, which is a critical issue for most applications. In these studies, we measured the oxidation potential of an array of Au and Ag nanoparticles attached chemically to glass/ITO electrodes, where the particle attachment occurred by diffusion to the electrode surface. For nanoparticles in the 1-4 nm diameter range, this leads to multiple oxidation peaks correlating to different size populations in disperse samples. In this presentation, we describe the size controlled assembly of nanoparticles on electrodes by electromigration. The size and amount of nanoparticles attached to the electrode surface strongly depends on the magnitude of the electrode potential during assembly and the direction of the voltage (negative or positive). Under certain conditions, we selectively assembled a large coverage of 1-2 nm diameter Au nanoparticles on the electrode with no evidence of aggregation. It is possible to selectively attach small nanoparticles in the presence of larger particles by controlling the voltage during assembly. The controlled assembly of small nanoparticles at large coverage on the electrode may find use in nanoparticle separation and purification and for electrocatalysis and sensing applications.

Keywords: Electrode Surfaces, Metals, Nanotechnology, Voltammetry

Application Code: Nanotechnology

Methodology Code: Electrochemistry
The utility of biological nanopores for the development of sensors has become a growing area of interest in analytical chemistry. Their emerging use in chemical analysis is a result of their reproducible control over nanoscale pore sizes with atomic level of precision and amenability to resistive-pulse type measurements. A single binding event causes a change in the flow of millions of ions across the membrane per second that is readily measured as a change in current with excellent signal-to-noise ratio. To date, ion channel-based biosensors have been limited to well-behaved proteins utilizing channels that remain in the open, conducting state, with little-to-no chemical specificity. In this talk, we explore the use of naturally occurring channels with inherent chemical specificity to build sensors. Specifically we utilize heat shock cognate 70 (Hsc70) to quantify the presence of adenosine triphosphate (ATP). Because Hsc70 forms ATP-dependent multiple conductance states we introduce the measurement of ‘charge flux’ across the lipid bilayer membrane to quantify ATP. We believe that monitoring protein-induced charge flux across a bilayer membrane represents a universal method for quantitatively monitoring ion-channel activity. Furthermore, we have transitioned measurements of Hsc70 to the glass nanopore membrane to create robust sensors with long-term stability.

Keywords: Bioanalytical, Biosensors, Electrochemistry, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Over the past decades, ultramicroelectrodes and nanoelectrodes have found wide applications in bioimaging and biomedical analysis. However, they have not been used in the fabrication of folding- and dynamics-based electrochemical biosensors. This presentation will highlight our recent advancement in employing gold-modified recessed platinum (Pt) nanoelectrodes in the fabrication of both stem-loop (SLP) and linear probe (LP) electrochemical DNA (E-DNA) sensors. We have successfully fabricated Pt nanoelectrodes with a radius less than 10 nm using an optimized laser pulling technique. Prior to sensor fabrication, the nanoelectrode is electrochemically etched to create a recessed nanopore, followed by electrodeposition of gold into the nanopore using either cyclic voltammetry or constant potential amperometry. Both techniques enable controlled deposition of gold into the nanopore, resulting in a nanostructured gold electrode with a well-defined surface area. In addition, we have systematically optimized the experimental condition for DNA probe immobilization and target interrogation. Both SLP and LP E-DNA sensors respond well to the full complementary target DNA. The extent of signal reduction is similar to that observed with sensors fabricated on macroscopic gold disk electrodes. While the application of these electrodes for real time biosensing in living cells requires further investigation, our results clearly show that these gold-modified nanoelectrodes can be used as substrates for this class of electrochemical biosensors.

Keywords: Biosensors, Electrochemistry, Electrode Surfaces, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Electrochemistry at Nanoscale Structures

Well-controlled array of nanopore structure with all components of battery is described. This nanopore battery structure provides a test-bed for examining the ion transport limits in nanostructured storage and may bring a new opportunity for an ultimate miniaturization in energy storage. We report a nanobattery comprised of nanotubular electrodes and electrolyte confined within an anodic aluminum oxide (AAO) nanopore as an “all-in-one” nanopore device. The nanoelectrodes include Ru nanotube current collectors with V2O5 storage material on top of the Ru to form a symmetric full storage cell, with anode and cathode separated by an electrolyte region. The V2O5 is prelithiated at one end to serve as anode while pristine V2O5 at the other end serves as cathode, so that the battery can be asymmetrically cycling between 0.2V and 1.8V. Capacity retention of this full cell at high power (relative to 1C values) is 95% at 5C and 46% at 150C. At 5C rate (12 min charge-discharge cycle), 81.3% capacity remains after 1000 cycles. The all-in-one nanopore battery poses an extreme case of highly confined organic electrolyte environments in ultrasmall batteries and provides a valuable data source for fundamental modeling to understand and quantify a science of “nanoionics” – a mesoscale phenomenon resulting from dense packing of ion storage nanostructures. In addition, upon extensive interest in creating more complex fully interconnected 3D porous film as a materials scaffold, understanding and manipulating the structure of AAO is a critical step for developing new techniques for the rational fabrication of 1D and 3D nanomaterials. A good understanding of the factors which impact structural control and the ability to combine multiple modification techniques in a single structure may open pathways to new structures with a wide variety of applications.

Keywords: Electrochemistry, Energy, Materials Science, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Electrochemistry
Infrared Spectroscopy (Well Beyond) the Diffraction Limit

Expanding Applications for Nanoscale Infrared Spectroscopy

AFM-IR, the combination of atomic force microscopy and infrared spectroscopy, has provided a dramatic improvement in the ability to chemically characterize materials at the nanoscale. The AFM provides extremely high spatial resolution, but historically has been without broadly applicable techniques for chemical analysis. Infrared spectroscopy, on the other hand, has excelled at chemical analysis, but has spatial resolution limited to the scale of many micrometers. The combination of AFM and infrared spectroscopy provides nanometer scale spatial resolution and robust chemical analysis in a single platform. The AFM-IR technique has recently been improved to enable chemical imaging and spectroscopy on a much more diverse range of samples, including thick and opaque samples. Additionally, the AFM-IR sensitivity has been dramatically improved to enable spectroscopic measurements on extremely thin films, down to single polymer lamellae, lipid membranes and even self assembled monolayers. This presentation will explore expanding applications of AFM-IR in the materials and life sciences including polymers, composites, thin films, semiconductors, biological membranes, cells, and biominerals.

Keywords: Infrared and Raman, Microspectroscopy, Vibrational Spectroscopy
Application Code: Polymers and Plastics
Methodology Code: Vibrational Spectroscopy
Infrared Spectroscopy (Well Beyond) the Diffraction Limit

Introducing Nano-FTIR – Imaging and Spectroscopy at 10nm Spatial Resolution

Neaspec’s near-field optical microscopy systems (NeaSNOM) allow to overcome the diffraction limit of light enabling optical measurements at a spatial resolution of 10nm.

Scattering-type Scanning Near-field Optical Microscopy (s-SNOM) [1] employs an externally-illuminated sharp metallic AFM tip to create a nanoscale hot-spot at its apex. The optical tip-sample near-field interaction is determined by the local dielectric properties (refractive index) of the sample and detection of the elastically tip-scattered light yields nanoscale resolved near-field images simultaneous to topography.

Development of a dedicated Fourier-transform detection module for analyzing light scattered from the tip which is illuminated by a broadband laser source enabled IR spectroscopy of complex polymer nanostructures (nano-FTIR) [2]. Identification of individual contaminants has been demonstrated. Other applications show characterization of embedded structural phases in biominerals [3] or organic semiconductors. The patented modular system design allows for tailored system configurations where the ultimate spectral coverage can be achieved by using synchrotron-based broadband IR light sources [4]. Use of material-selective frequencies for near-field measurements enable to fully characterize local morphology of organic semiconductors [5] or to analyse individual protein complexes at the nanoscale [6].


Keywords: FTIR, Materials Characterization, Nanotechnology, Vibrational Spectroscopy

Application Code: Nanotechnology

Methodology Code: Vibrational Spectroscopy
Infrared Spectroscopy (Well Beyond) the Diffraction Limit

Synchrotron Infrared Nano-Spectroscopy

By combining scattering-scanning near-field optical microscopy (s-SNOM) with mid-infrared synchrotron radiation, synchrotron infrared nano-spectroscopy (SINS) enables molecular and phonon vibrational spectroscopic imaging, with rapid spectral acquisition, spanning the full mid-infrared (500-5000 cm\(^{-1}\)) region with nanoscale spatial resolution. This powerful combination provides access to a qualitatively new form of nano-chemometric analysis with the investigation of nanoscale, mesoscale, and surface phenomena that were previously impossible to study with IR techniques. We have installed a SINS end-station at Beamline 5.4 at the Advanced Light Source (ALS) at Lawrence Berkeley National Laboratory, making the s-SNOM technique widely available to non-experts, such that it can be broadly applied to biological, surface chemistry, materials, or environmental science problems. We demonstrate the performance of synchrotron infrared nano-spectroscopy (SINS) on semiconductor, biomineral and protein nanostructures, providing vibrational chemical imaging with sub-zeptomole sensitivity.

Keywords: FTIR, Infrared and Raman, Microspectroscopy, Vibrational Spectroscopy

Application Code: Nanotechnology

Methodology Code: Vibrational Spectroscopy
Near-field superlenses [1] have the ability to break the diffraction limit due to the resonance of surface plasmon (or phonon) polaritons (SPPs) and enable applications like optical lithography and near-field imaging. Combining the superlens with scattering-type-near-field optical microscopy (s-SNOM) [2] enabled subsurface imaging of metallic nanostructures [3]. Up to now, the practical application of the superlens has been limited by its narrow bandwidth and complicated fabrication. We show our latest results on two new superlensing concepts using natural layered 2D-materials that overcome these limitations: Graphene and hexagonal boron nitride (hBN): Graphene, a one-atom-thick planar sheet supporting surface plasmons at frequencies in the infrared (IR) and terahertz (THz) spectral range [4,5]. In contrast to conventional superlenses, the nonresonant operation of our so-called “Graphene-Lens” (GL) [6] provides the advantages of a broad intrinsic bandwidth, a low sensitivity to losses and the possibility for continuously frequency-tunable imaging while maintaining a good subwavelength resolution of better than $\lambda/10$. We will show first experimental evidence for graphene superlensing [7]. Finally, we will present our latest subwavelength imaging results using thin layers of hBN, a material that supports surface phonon polaritons [8] and exhibits exciting new subwavelength imaging properties due to hyperbolic dispersion.

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Keywords: FTIR, Microscopy, Microspectroscopy, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Surface Analysis/Imaging
Mid-infrared absorption spectroscopy in the “molecular fingerprint” region is a powerful technique for chemical analysis. The mid-infrared photoexpansion nanospectroscopy was developed recently to measure absorption fingerprint spectra of samples on nanoscale [1]. In this technique, light absorption is detected by measuring associated sample thermal expansion with an atomic force microscope (AFM). Initially, this approach required high-power laser pulses to induce sample heating by 10-50 degrees and sample thickness of at least ~100 nm to produce detectable signal. We have recently demonstrated that the sensitivity of this technique may be improved dramatically to enable nanoscale spectroscopy of samples as thin as molecular monolayers [2]. This was achieved by adjusting the repetition frequency of low-power light pulses from tunable semiconductor quantum cascade lasers to match the mechanical vibrational frequency of the AFM cantilever and by employing tip-enhancement of the optical field below a sharp gold-coated AFM tip. We will discuss the latest development in sensitivity of this technique and present the first results of photoexpansion nanospectroscopy of samples in aqueous environment that requires mitigation of liquid damping of cantilever vibration and absorption of infrared light by water.

This project was supported by the Welch Foundation grant F-1705 and STTR program from the DOE.

Infrared Spectroscopy (Well Beyond) the Diffraction Limit

Polarized AFM-IR Studies of Anisotropically Oriented Polymers in Films and Nanofibers

The combination of atomic force microscopy (AFM) and infrared spectroscopy (IR) is a powerful tool that provides chemical, conformational and molecular orientation information at a spatial resolution of 50-100 nm. Using an AFM-IR (Anasys) instrument we have explored the correlation between structure, processing and chain orientation/crystallinity and tested the hypothesis that improved chain orientation in electrospun nanofibers can increase modulus and tenacity even while crystallinity is disrupted by appropriate addition of longer side-chain comonomer compositions. Polymer nanofibers and films have been produced with a wide range of crystallinity and molecular orientation. They have been characterized using polarized AFM-IR spectroscopy and imaging in order to assess the role of processing on orientation and morphology. This talk will highlight the use of AFM-IR to characterize molecular orientation and crystallinity changes as a function of side-chain comonomer concentration and illustrate the importance of obtaining rapid spatial spectroscopic images in inhomogeneous materials.

Keywords: Imaging, Instrumentation, Nanotechnology, Vibrational Spectroscopy

Application Code: Materials Science

Methodology Code: Vibrational Spectroscopy
Using infrared (IR) near-field nano-imaging (s-SNOM) and nano-spectroscopy (nano-FTIR), we report direct visualization of spontaneous structural and electronic phase transition and phase separation in vanadium dioxide (VO2). We show that s-SNOM and nano-FTIR are extremely powerful tools to study various types of phase transition materials such as VO2 single crystals and highly oriented VO2 films with epitaxial strain. The local strain induced by the substrate not only triggers spontaneous phase separation with facet dependent mesoscopic patterning in VO2 films but also leads to intermediate electronic and lattice states that are intrinsically different from the bulk single crystals'. This is clearly demonstrated by sampling the IR spectroscopic responses of electrons and phonons in VO2 with ~20nm resolution. I will also discuss the future applications of s-SNOM and nano-FTIR to many classes of transition metal oxides with low temperature phase inhomogeneities and magnetic orderings at microscopic scale.

Keywords: FTIR, Imaging, Infrared and Raman, Surface Analysis
Application Code: Nanotechnology
Methodology Code: Vibrational Spectroscopy
Critically ill patients in the intensive care units (ICU) commonly experience complications such as sepsis, delirium, acute lung injury (ALI) and acute kidney injury (AKI). These complex syndromes are all associated with systemic infections and lead to increased mortality. Currently these disorders are diagnosed through observation of clinical symptoms that are a result of the illness, meaning that treatments are administered reactively rather than proactively. Common to all of the aforementioned disorders is systematic inflammation, therefore biomarkers of emergent inflammation, namely pro- and anti-inflammatory cytokines, might offer a pre-symptomatic glimpse into latent syndrome. Cytokines help regulate inflammation through an intricate network of complementary and contradictory interactions that activate and deactivate different cohorts of immune cells. The complexity of these interactions necessitate a multiplexed approach to monitoring cytokine expression levels, and therefore panel-based diagnostic methods are needed to fully implement inflammatory-based diagnostics in a clinical setting. In response to this challenge, we have developed a powerful biomolecular analysis platform based on arrays of chip-integrated silicon photonic biosensors. Using this technology we have constructed a detection panel to simultaneously monitor fourteen serum-based inflammatory biomarkers and are correlating dynamic expression levels with the clinical outcome of patients being treated in the intensive care unit (ICU) at a local hospital. We expect that the biomarker trajectories of individual patients will reveal prognostic patterns suggesting the onset of complex clinical disorders.

This research was supported by the National Science Foundation Grant Award #1214081. WWS is supported by American Heart Association Predoctoral Fellowship.

Keywords: Bioanalytical, Biosensors, Clinical Chemistry, Immunoassay
Application Code: Biomedical
Methodology Code: Sensors
Solid contact ion-selective electrodes (ISEs) are multilayer structures, which have an intermediate layer between the ion-selective membrane and the electron conductor. The intermediate layer is used to improve reproducibility and stability of these electrodes compared to the coated wire electrode in which the ion selective membrane is in direct contact with the electron conducting substrate electrode. The influence of the intermediate layer composition and/or the electron conductor on the first equilibration time so far has not been studied systematically. The equilibration time is especially important for single use sensors and sensors implemented in point-of-care devices.

In this work we report equilibration times for solid contact ISEs prepared with (Poly(3,4-ethylenedioxythiophene) Polystyrene sulfonate – PEDOT(PSS)) conduction polymer as intermediate layer. The equilibration time is defined as the time interval from the first solution contact of the electrode and the time when the potential drift drops below a threshold value (0.3 mV/min). We have found that the second and third equilibration times are shorter for electrodes, which were kept dry between experiments. In addition, we found significant differences between the equilibration times of solid contact ISEs built on platinum, gold and glassy carbon substrates. The equilibration times were longer using Pt substrate. To trace the source of the unexpected differences in the equilibration times the structure of PEDOT(PSS) on Au and Pt surfaces were studied by X-ray photoelectron spectroscopy, and scanning electron microscope and the potential – time traces of PEDOT(PSS) coated Au and Pt electrodes were recorded.

Keywords: Biosensors, Electrochemistry, Electrodes, Ion Selective Electrodes
Application Code: Biomedical
Methodology Code: Sensors
Nonspecific binding onto surfaces is an issue that often prevents the successful adoption of some advanced diagnostic technologies. We report a versatile biopolymer-based, antifouling hydrophilic coating material for enhanced diagnostic applications. A chemical modification strategy of hyaluronic acid (HA) with the thermoresponsive polymer poly(di(ethylene glycol) methyl ether methacrylate) (PMEO2MA), a polymer with a lower critical solution temperature of 26°C in water, yielded an active coating onto a broad range of biomaterials without relying on the chemical functionalization of the surface. The polymer PMEO2MA was grafted onto HA to allow reversible formation of biopolymer coatings. Antibody conjugation of the obtained hydrophilic surface then yielded selectively bioactive, nonfouling surfaces (HA-PMEO2MA/HA-Ab). We demonstrate that the obtained surface significantly reduces non-specific binding to the surface and enhances detection levels of targeted markers. Detection sensitivity of inflammation marker TNF in serum protein-competitive samples was established using enzyme-linked immunosorbent assay (ELISA) and compared against biolayer interferometry (BLI) detection method to gain kinetic insights. The results showed superior performance of the HA-coated surfaces as compared to the non-coated ELISA plates in protein-competitive samples with error percentages of 5% and 15% respectively. While ELISA is the gold standard in protein detection, HA coatings enhanced detection by better preventing nonspecific binding to the matrix, as well as the simplicity of the coating process, and, as a result, lends itself as a viable option for more versatile and complex platforms.
A facile and robust method using redox active nanoparticles of cerium oxide to quantify enzyme activity in a single step procedure is reported in this presentation. The method takes advantage of redox changes of nanoceria surface after interaction with products of the enzyme catalyzed reaction, resulting in charge transfer complexes with very strong absorption characteristics. The developed assay is easy-to-use, robust and cost effective and does not require labeled reagents, secondary enzymes or soluble dyes. The assay can eliminate multistep procedures and minimize problems associated with the poor stability of substrates and enzyme labels of conventional enzyme assays. The assay has been adapted to both colorimetric paper and electrochemical sensing platforms and has demonstrated functionality for selected enzyme detection in human serum. This sensing concept can find wide applications as a general approach for improving sensitivity and simplifying detection schemes of colorimetric bioassays, e.g. enzyme, gene, immune and aptamer assays and related affinity sensing methods.
Since 2001 “early, goal directed therapy” is the accepted protocol for treatment of shock. The protocol essence is careful monitoring of metabolic parameters (venous carbon dioxide (CO2) and hematocrit) and adapting treatment to their changes. Emanuel Rivers demonstrated mortality decrease from 47% to 31% by following this protocol.1

A previously unexplored metabolic parameter, urine CO2, may also indicate prognosis and confer benefit to monitoring. Researchers have shown large differences in urine CO2 between healthy controls (mean urine pCO2 of 43mmHg) and hemodynamically unstable patients (mean urine pCO2 of 79mmHg).2 This discrepancy is likely from decreased splanchnic blood flow occurring in shock, where blood CO2 builds-up and diffuses into urinary bladder.

To investigate utility of urine CO2 as a prognostic tool for shock we have built a wall-jet type flow through manifold, which allowed continuous monitoring of pCO2 levels in urine samples, collected from a Foley catheter, with intermittent calibrations. As a pCO2 probe a commercial Severinghaus CO2 sensor with a silicon gas permeable membrane was used (Orion Research). Standard solutions were prepared by saturating buffer solutions with medical grade CO2 gas mixtures as well with an Instrumentation Laboratory tonometer.

In preliminary experiments we showed measurement set up and commercial CO2 sensor adequate for monitoring CO2 levels in quality control and human urine samples and developed a protocol for collecting urine samples from the Foley catheter with no CO2 loss. Next we will test the difference in CO2 content between patients in septic shock and healthy controls. This study is the first step of knowing the prognostic benefit of monitoring urine CO2 in septic shock.

Abstract Text
A comprehensive analysis of human metabolites provides significant information on the status of the metabolism with regard to diseases, medication, nutrition or even success of a therapy. However, the proven laboratory analytical methods require in general invasive sampling, transportation to the lab for analysis and finally transfer of the results to the physician in the clinic. As a consequence of this time consuming procedure, in many cases the initiation of a specific therapy is delayed with negative effects for the patient- e.g. higher lethality - and higher cost for the health system. Therefore, there is need for rapid on-site analytical tools for the detection of relevant metabolites in many fields in the health system. Using ion mobility spectrometry with rapid gas-chromatographic pre-separation and non-invasive sampling of e.g. human breath or volatiles from sweat it could be demonstrated, that on-line anesthesia control is possible as well as diagnosis and therapy control in nephrology. This is possible using compound and pattern data bases and a suitable data evaluation and interpretation software. Furthermore, the identification of relevant human pathogen bacteria and fungi is possible after less than 24 hours which could be used for applying a specific antibiotics therapy in good time.

Keywords: Clinical Chemistry, Clinical/Toxicology, Medical, Pattern Recognition
Application Code: Biomedical
Methodology Code: Chemical Methods
Fabrication of A Novel Fiber-Optic based Single-Cell pH Sensor

Comprehensive understanding of a single cell in response to its biological environment and stimuli is the foundation of many biomedical research fields, including drug development, nanotoxicity study, biomarker discovery, cancer diagnosis and treatment, and many other areas. One of the most important parameters is the intracellular pH changes during its exposure to the external stimuli. However, there currently is no such a probe available to perform measurement of intracellular pH without interrupt cellular activities. In this study, a novel tapered fiber-optic and reflection based pH sensor was designed and fabricated for both extra- and intra- cellular pH measurement, providing real-time pH data in mediating a variety of cell responses to external stimuli. The new pH sensor is easy to operate, responds fast, and has high resolution and sensitivity. Real-time sensing capability can be acquired with a response time of 20±5 seconds. A detection resolution of 0.02 - 0.05 pH unit was achieved with good repeatability and reproducibility. The detailed results will be presented at the conference. This project was supported by National Institute of Health (1R21GM104696-01).
Biomedical Sensors

Evanescent Wave-Based Microfluidic Capillary Biosensor

Optical fibers and capillary waveguides have been widely used in laboratory environments for biosensor applications; however, these biosensors have operational and design issues caused by several problems, including fragility, integration, and alignment of light and signals from optical fibers or capillaries, that prevent effective point-of-care usage. To address the challenges associated with optical fibers and capillary biosensors, we report an evanescent wave-based microfluidic capillary fiber-optic biosensor (MCFOB). As shown in the Figure below, the sensor combines capillary biosensors with microfluidic technology by embedding capillaries in a PDMS microfluidic device. Both capillaries and the microfluidic device serve a dual role within the device that enables integration and strengthen capillaries. The capillaries function as an optical element and a reagent vessel, while the microfluidic device serves as a cladding and multichannel for multiplexing. Since the geometry of capillaries has a defined volume, driving the reagent and sample is much easier and does not require complex tools such as pipettes. The alignment issues were addressed by using a CCD camera microscope and designing the capillary array in the plane. This makes detection and alignment from one capillary to another efficient. C-reactive protein (CRP), one of the biomarkers associated with inflammation and other diseases such as traumatic brain injury (TBI), was used to demonstrate the functionality of the MCFOB. A three-channel MCFOB was employed to detect CRP with a limit of detection of 7.6 ng/ml while the normal level of CRP in blood serum is around 640 ng/ml.

Keywords: Biosensors, Fiber Optics, Immunoassay, Lab-on-a-Chip/Microfluidics
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Many different electrochemical and electrophysiological techniques are available to study neurochemical systems which can elicit information regarding cellular activity, transmitter release, or receptor identity. Compared to their isolated use, incorporation of multiple techniques within one system, termed multi-modal, permits a broader characterization of the neurochemical network under investigation. For example in contrast to studies utilizing a single mode, multi-modal data can show simultaneous pre- and post-synaptic behavior which provides stronger evidence and enhances relationships between observed phenomena. In this approach we demonstrate multi-modal characterization in brain slices by first incorporating simultaneous intra- and extracellular electrophysiological recordings. Action potentials were evoked from medium spiny neurons (MSNs) in the rat dorsal striatum by intracellular current injections through a patch pipette. Cell spiking measured by the patch pipette was compared to single units recorded on a carbon-fiber microelectrode at various distances from the cell body to give a spatial profile for the extracellular electrophysiological signals. Secondly, iontophoresis was used to deliver dopamine to a cell in order to determine the cell type (D1 vs. D2) by the electrophysiological response, demonstrating how the combined techniques are useful for determining cell or receptor identity. Lastly, electrophysiological and electrochemical information were obtained simultaneously during electrically stimulated dopamine release. Oxidation of dopamine was performed on a carbon fiber to determine the release concentration from striatal dopamine terminals while the electrophysiology of MSNs innervated by the terminals was recorded.

Keywords: Electrochemistry, Method Development, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Huntington’s disease (HD) is a fatal, genetic, autosomal dominant neurodegenerative disorder. Although HD’s primary symptoms include motor deficits, patients with HD are also at increased for depression, heightened anxiety and aggression, as well as other mood disorders. Since serotonin has been implicated in mood disorders, including depression, it is important to study serotonin release in HD. Fast-scan cyclic voltammetry (FSCV) is an electrochemical technique with high spatial and temporal resolution that, when used with carbon-fiber microelectrodes in brain slices, can be used to elucidate information about neurotransmitter release. Here, we employ FSCV in order to measure serotonin release in HD. Electrically stimulated serotonin release was measured in brain slices from R6/1 and R6/2 model HD mice. We find that, in both models, release is significantly impaired. Moreover, using pharmacological manipulations, we quantified reserve pool serotonin in the dorsal raphe nucleus. Findings from these experiments will also be discussed.
In Vivo Electrochemical Studies of Salicylates in Rat Brains

Salicylates (SA), such as Acetylsalicylic acid which is also known as aspirin, and its active metabolite, salicylic acid, are wildly used to treat moderate pain, inflammation and fever. However, consumption in large doses of SA can have serious side effects, including internal bleeding and dizziness. The mechanism of the causes of these side effects by SA are still not clear. Despite various reported analytical methods for identification and qualification of SA, its characteristic features in the body have not been fully studied. In this study, we present the electrochemical characteristics of SA in the brain of anesthetized rats using fast-scan cyclic voltammetry (FSCV). FSCV coupled with carbon fiber microelectrode possesses high temporal and spatial resolutions which allows us to monitor sub-second change of the SA concentration in small brain regions. Here we demonstrate the stability and sensitivity of this method in the brain. With the optimized conditions, the minimum detected concentration was 50 nM and a linearity range was from 200 nM to 20 µM. The response sensitivity was largely unchanged. We demonstrate for the first time that the FSCV coupled with a carbon-fiber microelectrode yields a sensitive, reproducible and stable response for the salicylates during in vivo electrochemical measurements.

Keywords: Biosensors, Electrochemistry, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Hydrogen peroxide, whose role in the complex environment of the brain is not well understood, has been implicated in the slow destruction of dopaminergic neurons in Parkinson’s disease (PD). This neurodegenerative disease affects more than a million people in America, creating a critical need to identify the mechanisms through which hydrogen peroxide interacts with dopaminergic neurons. Real-time in vivo detection of this analyte has recently been described using fast-scan cyclic voltammetry at carbon-fiber electrodes. However, selectively identifying hydrogen peroxide from interferents such as adenosine and pH shifts remains a challenge. Additionally, some chemical agents used to pharmacologically verify the presence of hydrogen peroxide production in the brain, such as mercaptosuccinic acid, also have a similar oxidation peak as that of the target analyte, further convoluting the characterization of hydrogen peroxide dynamics in the brain.

We have addressed these problems by fabricating a hydrogen peroxide-selective electrode. 1,3-phenylenediamine (mPD) was electrodeposited onto the surface of a carbon-fiber electrode to render it sensitive to hydrogen peroxide fluctuations and pH shifts, but not other analytes. Since pH changes generate a well-characterized and distinct voltammogram, they can easily be removed from the signal using principal component regression, to reveal a straightforward quantifiable electrochemical signal due solely to the oxidation of hydrogen peroxide. This technology was fully characterized on glass-insulated electrodes for acute implantation, as well as electrodes designed for chronic implantation. The work presented herein will facilitate the selective detection of hydrogen peroxide, opening the door for further elucidation of the neurodegenerative role it plays in PD, as well as other neuropathies involving oxidative stress.

Keywords: Chemically Modified Electrodes, Electrochemistry, Microelectrode, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Electrochemical Approaches to Understanding Brain Function

Voltammetric Measurement of Rapid Dopamine Dynamics During Continuous Intracranial Self-Stimulation

Fast-scan cyclic voltammetry (FSCV) is a useful tool for the sub-second detection of catecholamines in vivo. When monitoring dopamine neurotransmission in freely-moving animals, both high sensitivity and high temporal resolution are desirable. The traditional waveform used, which scans between -0.4 V and 1.0 V, has high temporal resolution, but only moderate sensitivity. Modifying the anodic limit of the voltammetric sweep can increase sensitivity towards dopamine. Dopamine adsorbs to the surface of carbon-fiber microelectrodes under physiological conditions. At more positive potentials, overoxidation of the carbon-fiber surface produces negatively charged carbonyl groups that promote adsorption of dopamine and other physiological cations. However, adsorption and desorption kinetics slow the time response of the voltammetric measurement, resulting in diminished temporal resolution, leading to a commonly observed tradeoff between sensitivity and temporal resolution.

In order to pair sub-second dopamine changes with behavioral information, it is crucial to understand the benefits and limitations of the voltammetric waveform employed. It is our aim to investigate the effect of several waveforms on the dopamine signal while a rat is pressing a lever for intracranial stimulation. Data collected with anodic limits of 1.0, 1.3, and 1.4 V reveal contrasting dopamine signals during this behavior. Additionally, pharmacology will be used to further investigate the underlying regulatory processes behind dopamine dynamics during ongoing intracranial self-stimulation.

Abstract Text

Keywords: Electrochemistry, Microelectrode, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Brain microdialysis allows the collection of a variety of substances and can be paired with numerous detection methods, thus the versatility of this technique has led to its widespread use. While microdialysis is an extremely important technique, it is limited by the immune response occurring around the probe. The severity of the response changes with time and impacts the concentrations of evoked dopamine measured with the probe. This results in a discrepancy in the concentrations sampled from the probe compared to the concentrations in the surrounding tissue. Voltammetry next to the probe has illustrated this problem by showing a decreasing gradient of evoked dopamine the closer the electrode is placed to the probe. In an acute study, dexamethasone, an anti-inflammatory drug, reduced the immune response and protected evoked dopamine directly next to the probe. The goal is further investigate the relationship between the concentrations detected with voltammetry in the striatum to those sampled with the microdialysis probe. To directly compare to the responses next to the probe, voltammetry was used at the outlet of the microdialysis probe in a 4 and 24 hour study with and without dexamethasone. After 4 hours the outlet concentration was not significantly affected by dexamethasone. However, after 24 hours dexamethasone preserved the evoked response while the concentration significantly decreased without the anti-inflammatory drug. The positive effects observed after 24 hours with dexamethasone prompted further investigation into longer terms studies.
Clinical results of monitoring brain injury patients have characterised rapid metabolic changes, notably spreading depolarisations (SDs) in the injured human brain [1]. During SD events, glucose levels decrease and lactate levels increase and event repetition can drive the local glucose levels down, possibly to a level where the tissue is no longer viable [2].

We have designed a bedside monitoring system to analyse the dialysate from a microdialysis probe implanted in the “at-risk” brain tissue. This uses a microdialysis probe connected to an online microfluidic analysis platform. Combined needle electrodes, designed to fit into an analysis chamber on a microfluidic chip [3] are fabricated into amperometric sensors by first applying a protective mPD film formed by electropolymerisation. An enzyme layer is added by dip coating the electrode in a mixture of BSA, glycerol, PEG-DE and specific enzyme and drying at 55°C for 2 hours [4]. Sensors for glucose, lactate and pyruvate will be discussed. A common problem is calibration and an automatic system will be described, eliminating the need for a specialist to constantly be nearby the equipment. The system has been tested and is now located at the hospital awaiting patient access.

We thank the Wellcome Trust / DOH HICF fund.

Aversive and pleasurable stimuli have been shown to drive dopamine (DA) signaling in the nucleus accumbens (NAc). Increased DA signaling in the nucleus accumbens (NAc) has been demonstrated following pain offset, suggesting that this region is important for pain relief. To date, changes in NAc DA efflux have not been extensively investigated using voltammetric methods in freely moving animals or demonstrated over extended periods of time. Pain is expected to affect both tonic (steady-state concentration in the extracellular space) and phasic (transient changes induced by burst firing) activity of dopaminergic neurons. Recently, we have developed fast-scan controlled-adsorption voltammetry (FSCAV) and coupled this technique to fast-scan cyclic-voltammetry (FSCV) to enable multitemporal analysis of dopamine signaling with a single sensor. This single sensor strategy and the stability of PEDOT:Nafion coated electrodes was validated with measurements of tonic and phasic dopamine signaling over a 4 week period at 1, 7, 14, 21 and 28 days after probe implantation in Sprague-Dawley rats. Electrode placement was then verified post-hoc with histology. Lastly, using FSCAV the degree of tissue damage over the 4 week period was quantified using a previously validated implicit finite difference model. Detection of NAc DA in freely moving animals over extended time periods may allow for investigation of pain without complications of anesthetic agents.

Keywords: Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Monolayer protected gold nanoparticles (NPs) have recently been proposed as possible components of improved photovoltaics. The passivating layer can create an electrical double layer on the NP surface, allowing NPs to serve as quantized double-layer (QDL) capacitors and store charge for such devices. In order to effectively implement nanoparticles in this field, it is important to understand the electron transfer properties of a variety of protecting ligands.

In this research, we determine the forward heterogeneous electron-transfer rate constant ($k_f$) of wired organic-soluble and water-soluble monolayer protected gold NPs using the scanning electrochemical microscope (SECM). Using SECM approach curves, we were able to determine the electron transfer rates of nanoparticles protected with a variety of ligands. By altering the monolayer composition through place exchange as well as changing the ligand charge by adjusting solution pH, we demonstrate effective means of modulating electron transfer rates.

Our results show that the electron transfer rate is largely dependent on the protecting monolayer composition and the charge presented by the ligand shell. By adding a relatively small amount of highly conductive molecular wires, we can dramatically increase the electron transfer rate. By changing the ligand charge on tiopronin protected gold NPs through the adjustment of solution pH, we show that the electron transfer rate is inversely proportional to pH and decreases dramatically as the ligands move from an uncharged species to a negatively charged one. This demonstrates charges on ligands inhibit the tunneling of the electron through the protecting monolayer and lowers the electron transfer rate.
Characterization of small colloidal nanoparticles (NPs) i.e. NPs less than 10 nm in diameter is an analytical challenge. We present ZnO quantum dots (QDs) as a case study for the electrochemical detection of small nanoparticles. Quantum confinement imparts different properties to ZnO from the bulk such as exceptional optical, electronic and magnetic properties. Currently this material has been used as a promising candidate in different technical fields that include applications of the QD colloids. ZnO QDs were synthesized according to the sol-gel method and were characterized by UV-Vis and fluorescence prior to the experiments (Chem.Commun. 2011, 47(43), 11921-3); the spectroscopic measurements were used to follow the stability of the materials. Different concentrations of ZnO quantum dots in CH[sub]3[/sub]CN were reduced at -2.40 V vs. Ag/AgCl at an electrochemically prepared Hg ultramicroelectrode on Pt. ZnO reduction is carried after collision with Hg microelectrode with the particle resident time at the Hg surface influencing the shape of the current transients. We present the detection of the QDs by the reduction of Zn[sup]2+[/sup] in ZnO. Current spikes and steps are observed likely due to different contributions from single nanoparticles and agglomerates at the Hg microelectrode. The number of moles of reduced Zn[sup]2+[/sup] was calculated from the stripping peak around -1.5 V vs. Ag/AgCl, which represents the oxidation of Zn[sup]2+[/sup] in Zn(Hg). The calibration curve plotted as a function of the reduced amount of Zn[sup]2+[/sup] vs. concentration of ZnO quantum dots is used to study the limit of detection of the technique.

Keywords: Characterization, Electrochemistry, Nanotechnology, Semiconductor
Application Code: Nanotechnology
Methodology Code: Electrochemistry
The metal galvanic exchange reaction has been utilized in recent years to synthesize pure metal and alloy nanomaterials with structures not easily obtainable by other methods. Examples include the synthesis of hollow nanotubes, nanoframes, nanorattles, and nanocages. These materials usually exhibit unique optical, electronic, or catalytic properties. While this reaction has been widely studied on various shaped metal nanostructures, there is little known about the effect of nanoparticle size on the galvanic exchange reaction. Our group recently showed that the oxidation potential for Au nanoparticles decreases by about 200 mV from bulk size to 4 nm in diameter and by another 600 mV for Au nanoparticles in the 1-2 nm diameter range. Since galvanic exchange involves the oxidation of one of the metals involved, it is reasonable that the reaction will be highly dependent on size, especially for very small sizes, where the negative oxidation potential shift is dramatic. In this presentation, we describe the galvanic exchange of Au nanoparticles attached to electrode surfaces with different concentrations of PtCl$_4^{2-}$ complex as a function of the Au nanoparticle size from 4 nm to 90 nm, while keeping the surface area of Au constant. We used voltammetry, scanning electron microscopy, and CO striping analysis to quantify the amount of Pt on the resulting AuPt alloy nanoparticles in order to determine the amount of exchange. The exchange reaction is highly size-dependent, showing a much larger amount of exchange occurring on Au nanoparticles smaller than 10 nm. The difference in reactivity is due to the negative shift in the oxidation potential of Au nanoparticles as size decreases. We discuss this unique reactivity for small metal nanoparticles and possible applications in catalysis and sensing.
Electrochemistry/Nanotechnology

Redox Nano-Titrations on Operating Water-Splitting Semiconductor Electrodes for the Quantification of Photogenerated Intermediates

The in situ characterization of the main photogenerated chemical intermediates, holes and hydroxyl radical, on operating semiconductor electrodes in aqueous media through their basic surface descriptors such as coverage, mobility, and spatial distribution, including the effect of surface perturbations, remains largely unexplored.

Here, we studied the water splitting and water oxidation reactions at n-doped strontium titanate electrodes. In our approach, carbon probes with radii few [micro]m to tens of nm were used in the context of scanning electrochemical microscopy feedback measurements to activate redox mediators capable of titrating reacting species on the operating semiconductors during UV illumination and external bias. Positive feedback curves were obtained in general during illumination, and this allowed to extract the overall rate of oxidation by both holes and hydroxyl radicals. Using the recently-introduced Surface Interrogation mode of SECM [1] for studying adsorbed intermediates, and in combination with simulation methods, we were able to decouple the reactivity of hydroxyl radicals and obtained their coverage and reaction kinetics. This result permits to weight the contribution of both surface species on photo-assisted oxidations. Furthermore, electrochemical imaging allowed to detect differences in the local surface reactivity on well-controlled crystal defects. Experimentally discerning the differences in the dynamics of holes and hydroxyl radicals on native and defective structures will open new avenues in the rational design, modification and efficient utilization of photoactive materials for environmental, energy and synthetic applications.


Abstract Text
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Keywords: Energy, Semiconductor, Surface Analysis, Titration
Application Code: Materials Science
Methodology Code: Electrochemistry
Composite corrosion coatings that increase hardness and slow the rate of corrosion at a reduced cost have been heavily studied due to the persistent problems occurring with corrosion. Electrodeposition has become an advantageous technique in manufacturing coatings because of reduced cost, convenience, and the capability of working at low temperatures. In the off-shore oil field industry, high corrosion resistance and hardness are needed to extend the lifetime of coatings due to exposure to high stress and salt environments. In marine environments, copper alloys are used to defend against biofouling of materials by inhibiting microbial induced corrosion (MIC). Copper ions are capable of eradicating viruses, algae, fungi, bacteria, and other microbes. A major copper alloy currently studied is Cu-Ni (90/10), which shows good protection in low flow to stagnant conditions. With the addition of montmorillonite into the Cu-Ni matrix, an increase in strength, adhesion, wear and fracture toughness of the coating occurs, which leads to an increase in corrosion resistance and longevity of the coating. These coatings were evaluated for composition and morphology using many different types of instrumental techniques including x-ray diffraction, atomic absorption spectroscopy, scanning electron microscopy, energy dispersive spectroscopy, atomic force microscopy, and nanoindentation. Also, electrochemical techniques including electrochemical impedance spectroscopy and Tafel analysis were used to evaluate the corrosion protection of the films. The overall corrosion resistance and hardness was improved with the films, which holds promise for the off-shore drilling environment in the oil and gas industry.

**Keywords:** Electrochemistry, Materials Science, Paint/Coatings, Surface Analysis

**Application Code:** Materials Science

**Methodology Code:** Electrochemistry
The present work has been carried out with the aim of quantitatively investigate the mass transport parameters of electrochemically facile systems such as hydrogen evolution reaction (HER), potassium ferri/ferrocyanide, and ruthenium hexaammine(III/II) in high concentrations. HER, as one of the most prominent reactions in renewable-energy technology occurs in highly concentrated media at a real hydrogen power station. At this high concentration, migration contributes to mass transport which in turn affects the overall rate of proton reduction and hydrogen generation. To the best of our knowledge, these effects have not been discussed at high concentration (> 0.1M) in the literature, in part because of limitations of conventional techniques.

Here, two electrochemical techniques, cyclic voltammetry and chronoamperometry, are used to measure the apparent rate constant and the diffusion coefficient of the electroactive species, in the presence and absence of supporting electrolyte and high analyte concentration on micrometer-sized and nanometer-sized electrodes experimentally and by simulation.

In the absence of a supporting electrolyte, changes in diffusion coefficients of electroactive species and steady state currents reveal the obvious effect of migrational component coupled with electron transfer due to the build-up of an electrical field within the electrode’s depletion layer.

More interestingly, chronoamperometric evaluation of D for highly concentrated ruthenium hexaammine(III/II) (Fig.1) and HER, as two reference systems can allow the accurate determination of electrode surface area in nanometer scale by providing larger diffusion-limited currents that do not require a highly sensitive potentiostat. Thus, electrode area characterization, a common challenge in nanoelectrode technology would be facilitated.

Keywords: Electrochemistry, Microelectrode, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Electrochemistry
Supporting electrolyte is commonly used in electrochemical experiments to minimize the contribution of electromigration transport of the redox-active species being studied. At low ionic strength, ion migration can contribute significantly to mass transport, promoting or impeding the movement of ions to the electrode surface. In addition, ion enrichment has been widely observed in charged nanopores and nanofluidic channels at low ionic strength to maintain electroneutrality. By taking advantage of the effects of both ion migration and ion accumulation, large current enhancements are achieved during cyclic voltammetry of Ru(NH$_3$)$_6$H$^{3+}$ at a recessed ring-disk nanoelectrode array without supporting electrolyte. Limiting current as a function of the concentration of Ru(NH$_3$)$_6$H$^{3+}$, exhibiting three distinct regimes, revealed strong dependence of ion accumulation and transport on ionic strength. Voltammetric response at low analyte concentrations is especially interesting, since ions accumulating in the nanopores results in dramatically increased limiting current compared to measurement in the presence of supporting electrolyte. As large as 100-fold current enhancements due to ion enrichment and migration effects adding to the ~20-fold enhancement arising from redox cycling produces a total current amplification of ~2000-fold compared to that obtained on a single microelectrode of the same total area.

Keywords: Electrochemistry, Electrodes, Lab-on-a-Chip/Microfluidics, Voltammetry
Application Code: Nanotechnology
Methodology Code: Electrochemistry
A magnetophoretic enrichment method was designed for improving signal response time and detection limits in the chronoamperometric observation of discrete nanoparticle/electrode interactions by electrocatalytic amplification. The strategy relied on Pt-decorated iron oxide nanoparticles which exhibit both superparamagnetism and electrocatalytic activity for the oxidation of hydrazine. A wet chemical synthetic approach succeeded in the controlled growth of Pt on the surface of FeO/Fe[3]/O[4] core/shell nanocubes, resulting in highly uniform Pt-decorated iron oxide hybrid nanoparticles with good dispersibility in water. The unique mechanism of hybrid nanoparticle formation was investigated by electron microscopy and spectroscopic analysis of isolated nanoparticle intermediates and final products. Individual hybrid nanoparticle collision events were detected in the presence of hydrazine electrochemical indicator using a gold microband electrode integrated into a microfluidic channel. The experimental nanoparticle/electrode collision rate correlates much more closely with simple theoretical approximation in comparison to previous related systems, due in large to the accuracy of the nanoparticle tracking analysis method used to quantify nanoparticle concentrations and diffusion coefficients. Further modification of the microfluidic device was made by applying a tightly focused magnetic field to the detection volume in order to attract the magnetic nanoprobes to the microband working electrode, thereby substantially increasing the relative frequency of chronoamperometric signals corresponding to nanoparticle adsorption events. With proper biomodification of nanoparticle and electrode surfaces, the presented magnetically enriched single-nanoparticle detection platform has the potential to develop into a practical lab-on-a-chip device for nucleic acid and protein sensing at ultratrace concentrations.
Environmental - GC/MS Techniques

Comparison of VOCs in Whole Blood From Burn and Smoke Victims With Other Populations by SPME GC-MS

Burn inhalation injury is difficult to diagnose, lacks effective treatment, and significantly increases patient mortality. The characterization of volatile organic compounds (VOC) in burn patients with smoke exposure may assist in future diagnosis and treatment of burn inhalation injury, and in turn, decrease related mortality. The purpose of this study was to characterize the VOC concentration levels of burn patients and compare these levels to estimates for certain U.S. subpopulations (e.g., cigarette smokers and nonsmokers). Blood samples were quantified by passive headspace sampling using solid phase micro-extraction (SPME) followed by GC-MS with select ion monitoring (SIM). Internal standard was added during sample preparation to compensate for analyte specific matrix effects, competition effects at the SPME fiber, and diffusion loss biases. The analysis of volatiles at low levels is easily influenced by contamination, so study design and sample collection must take into account the prevalence of VOCs in common lab materials. Because the compounds in question are volatile, care must also be taken to protect against sample loss. To prevent contamination bias or loss of VOCs, samples must be stored in pre-cleaned, airtight, blood collection tubes from sample collection to analysis. Our method possesses the sensitivity and specificity to distinguish among VOC exposure sources, including smoke from cigarettes and burning buildings. For example, mean blood benzene levels are significantly different (p-values <0.05) for nonsmokers (29 ppb), smokers (211 ppb) and smoke inhalation victims (910 ppb). This presentation will cover the importance of VOC biomonitoring, the analytical method described above, and a discussion of the results used to differentiate exposure sources.

Keywords: Environmental/Biological Samples, Gas Chromatography/Mass Spectrometry, SPME, Volatile Organic

Application Code: Environmental

Methodology Code: Gas Chromatography/Mass Spectrometry
There is an increasing need to measure both volatile and semi-volatile components in air samples as impact to human health concerns continue to be an issue. Until recently, the analysis of both volatile and semi-volatile components required two analytical methods. This presentation will demonstrate the ability to test both volatile and semi-volatile components in air using one analytical method.

Through rigorous investigation, a new sampling tube and method have been developed to monitor 1,3-Butadiene, Benzene, Toluene, Ethyl Benzene, Xylenes (BTEX) and the 16 EPA regulated polynuclear aromatic hydrocarbons (PAHs) in one air sample. It is advantageous to perform this in one analysis instead of two. Only one sample will need to be collected in the field instead of two which reduces sampling costs significantly. Using one method, EPA Method TO-17, instead of two methods EPA TO-13 and TO-15 enhances laboratory productivity, profitability, and safety. In addition, since the analysis can be accomplished with one method, less solvent and electricity is used, making it a “greener” analysis.

EPA Method TO-13 requires 24 hours of soxhlet extraction using 100-300mL of methylene chloride and a concentration step before the sample gets to the instrument for analysis. TO-17 requires no sample preparation when the samples get to the lab. The tubes are placed on the autosampler. After starting the thermal desorber, the instrument automates the process of desorbing the analytes from the tube, and injecting the sample into the analytical column for analysis and GC/MS results. This is a fast analysis driven by chromatography and not tube desorption.

This presentation will include additional benefits of TO-17 over TO-13 and TO-15. A brief overview of the thermal desorption process will be reviewed. The analytical results will be discussed including data from site samples.

**Keywords:** Air, Coal, Environmental, Gas Chromatography/Mass Spectrometry

**Application Code:** Environmental

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Environmental - GC/MS Techniques

Selective and Sensitive Detection and Quantification of Stockholm Convention Pops, Including Dioxins, Using Atmospheric Pressure Gas Chromatography MS/MS

Atmospheric pressure GC (APGC), first developed in the 1970s, has recently made a comeback as an alternative to high resolution EI-GC/MS and EI-GC/MS/MS. For many of the POPs on the Stockholm Convention, only the molecular ion is formed under APGC ionization conditions. By avoiding fragmentation, the sensitivity in MRM or SIR mode is enhanced. In this work, APGC with a high sensitivity tandem quadrupole mass spectrometer was used in MRM mode under "dry" N\textsubscript{2} conditions. Standards for dibenzo-p-dioxins and furans were obtained from Wellington Labs. Data were acquired with two transitions for each of the native compounds and their \textsuperscript{13}C analogues. The results demonstrated that the sensitivity achieved using APGC-MS/MS is comparable with high resolution GC/MS. Injection of a 1/10 dilution of calibration standard CSL of concentration range 10-100 fg/\mu l resulted in S/N from 10 to 65. A calibration curve was generated by analyzing 1/10 CSL up to CS4 standard. Coefficients of determination ($r^2$) for all compounds over the calibration range were all > 0.998. To test the sensitivity of the system, the lowest calibration point was diluted 1/10. The S/N for the first or second MRM ranged from 10 (10 fg TCDD/TCDF) to over 50 (other congeners 50-100 fg). Repeatability for n=10 injections of the CSL standard were all less than 10% RSD. The relative standard deviations ranged from 2.5 to 9.6%. In addition, ion ratio deviations were all less than 20% across the calibration range. These results meet the specification often set for high resolution GC/MS systems.

Keywords: Environmental, Environmental Analysis, Gas Chromatography/Mass Spectrometry, Instrumentation
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Halogenated dioxins and furans have long been persistent organic pollutants of environmental concern produced as byproducts from a variety of sources. It has become important to be able to detect a range of dioxins and furans at trace levels in a diverse range of matrices. In addition to increased sensitivity, accuracy and repeatability are also important for dioxin analysis of environmental samples. Historically, the preferred method of analysis has utilized GC-HRMS systems employing double-focusing, or “sector” based instruments that monitor specific mass-to-charge fragments for each target compound. In this study, an alternate method utilizing atmospheric pressure ionization gas chromatography (APGC) coupled to a triple quadrupole mass spectrometer (TQS) is described to allow identification of a more comprehensive list of compounds in a single run, as well as its use as a discovery tool.

Through an extensive comparison of samples analyzed using both methodologies, APGC-TQS analysis has demonstrated to be a comparable technique to sector-based HRMS analyses for trace level identification of dioxins and furans in environmental samples. Another benefit of this instrumentation is the ability to report a limit of detection on the single femtogram level. The method developed can simultaneously monitor for tetra- through octa- chlorinated dioxin and furan congeners, including the seventeen toxic congeners, in a single run. Furthermore, methods have been developed for identification of a variety of polybromo- and mixed bromo/chloro- dioxins and furans. Application of these methods will be demonstrated on a variety of sample matrices including sediment, fire debris, and human serum.

Keywords: Environmental Analysis, Gas Chromatography/Mass Spectrometry, GC-MS, Trace Analysis
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Polyaromatic hydrocarbons (PAHs) are compounds containing two or more aromatic rings and are known for their potential carcinogenic and mutagenic properties. They are typically formed during incomplete combustion of organic matter, as industrial byproducts, and in food processing. As such, they are compounds of interest in environmental food and beverage, and toxicological analysis. PAHs have become a target for many governments and regulatory agencies. US EPA method 610 is the benchmark method for PAHs, using nonpolar silicon based stationary phases operated at high temperatures. Separations on these phases are prone to coelution, specifically for the isomers of anthracene, chrysene, benzo(k)fluoranthene, and dibenzo (a,h)anthracene.

Ionic Liquids (ILs) are a new class of GC stationary phases that provide unique polar and highly polar selectivity with higher thermal stability compared to traditional GC phases with similar selectivity. Our previous work demonstrated that phosphonium ILs possess superior selectivity for PAHs than imidazolium IL-based phases. In particular, SLB-IL 59 has baseline resolution of isomeric pairs that are of interest including anthracene/phenanthrene, cyclopenta(c,d) pyrene, chrysene/triphenylene, and the benzofluoranthenes. Building upon these results, our goal has been to study the chemistry of other phosphonium IL analogues, attempting to improve thermal stability and decrease analysis time. Our secondary goal has been to study and optimize the GC and column parameters to create a robust method for PAH analysis.
Concern about environmental impact and the need to remain current with evolving regulation has driven many refineries and chemical manufacturers to adopt increasingly rigorous methods for measuring the composition of the gas mixtures headed to their flare stacks. Most recently, in the United States the EPA’s implementation of 40 CFR 60 subpart Ja has mandated the measurement of hydrogen sulfide, total sulfur, and hydrocarbon content in many refinery flares. Similar regulations have been enacted, or are in process in other countries and parallel industries.

Flare streams consist of a mixture of off gas from several plant processes, meaning that they are subject to wide variation in terms of components present and their relative concentrations. While gas chromatography has been employed in monitoring flare emissions for some time, process mass spectrometry is less common. The ability of a mass spectrometer to measure hydrogen sulfide, total sulfur, and hydrocarbon content (heating value) within the changing mixture of the flare stream provides the plant with a single-analyzer solution capable of measuring from the ppm level up to 100% without saturating the detector. Additionally, the linearity of analysis offers the potential for system validation using safe-to-handle standards. A quadrupole mass spectrometer was used to monitor a variety of flare gas mixtures. Release conditions were simulating and the concentration of H2S was monitored from ppm levels up to 100% to demonstrate accuracy, and linearity.
Fluorescence generation and quenching are two common approaches to RNA/DNA biosensing. Often fluorescence generation (signal-on) is considered to be more sensitive than quenching (signal-off) based mechanisms. However, sensitivity is not the only metric when considering optimal biosensor performance. To the best of our knowledge, no work has been done directly comparing the analytical figures of merit for a biosensor that can both generate and quench fluorescence. We have established such a comparison using a fluorescent miRNA biosensor recently developed in our group. The overall mechanism starts with the reporter molecule in a fluorescence-quenched hairpin configuration. Addition of a partially complementary probe opens the hairpin, resulting in the generation of fluorescent signal. Presence of the target analyte (miRNA) will displace the reporter as the analyte binds the probe. The reporter then folds back into the quenching hairpin. We will present a detailed comparison of the sensitivity, specificity, limits of detection, false signals, and signal to noise for a signal-off reporter-probe vs. a signal-on molecular beacon biosensor. Upon comparison of signal-on to signal-off, we found an order of magnitude lower LOD for signal-on mechanisms. However, the signal-off mechanism of the reporter-probe biosensor demonstrates higher selectivity than the signal-on molecular beacon. Given the signal-off biosensor achieves picomolar limits of detection with similar signal to noise as signal-on approaches, we conclude that for short nucleic acid sensing, signal-on does not offer any substantial advantages over signal-off approaches. Ongoing work is focused on using the reporter to image miRNA in cells and tissue.

Keywords: Biosensors, Fluorescence, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
A sensor for H2O2 was developed by enclosing the semiconductor nanocrystals (NCs) inside the hydrogel nanoparticles together with the metal-responsive dyes. Oxidation of the NCs by H2O2 causes NC dissolution, and the dissolved cations would trigger fluorescence from the fluorogenic dyes, making the nanogel particles emitting strong fluorescence. The reaction mechanism was investigated. The linear range was found to be from 0.1 uM up to 8 mM, with the detection limit of 0.08 uM. The sensor has good selectivity for H2O2 over other oxidative species. Its application in testing release of H2O2 from cancer cells has been explored.
# Distance Dependence of Fluorophore Quenching by Graphene Oxide with Rigid Spacer

The quenching property of graphene oxide (GO) was widely used for the fluorescence sensing and imaging. However, the quenching distance of GO to dyes were only investigated with the assistant of DNA strands, which might cause false distance measurements because of their flexibility on the surface of GO. Therefore, a rigid distance spacer, silica shell, between GO and dyes was used in this work. Organic dye was doped in the silica nanoparticles first, followed by the modification of another layer of silica shell with different thickness. Due to the electrostatic interaction between GO and positive charged silica nanoparticles, GO wrapped the silica nanoparticles when they are mixed together. Therefore, the distance between GO and organic dyes was adjusted by the thickness of the silica shell. The results showed that the quenching efficiency was higher than 50% when the distance was smaller than 7.1 nm. This quenching ability investigation of GO to dyes with distance-dependent manner would provide important information for the design of the fluorescent functional composite using GO in the future.

**Keywords:** Biomedical, Fluorescence, Nanotechnology

**Application Code:** Bioanalytical

**Methodology Code:** Fluorescence/Luminescence
Silica spheres have been used as a carrier for small molecules in the past few decades because of their intrinsic features, including the relative ease of surface modification, which has permitted application in biological/biomedical areas. Many types of fluorophores (e.g., dyes and metal-based quantum dots) have been incorporated into the silica skeleton/surface to prepare luminescent particles. One major problem with dyes is their leakage from the silica particles, hampering the long-term stability of the imaging system; this is also the case with metal-based quantum dots. The chemical stability and photostability also need to be improved. Fluorescent carbon nanodots (C-dots) have been gaining increasing interest as a new type of fluorescent material. Their excellent biocompatibility, chemical inertness, and photostability make them ideal alternative for traditional organic dyes or metal-based quantum dots. In this study, sub-micron fluorescent silica particles were synthesized by a modified Stöber method. Fluorescent C-dots made from bottom-up synthesis were used as fluorescent precursor. Experimental parameters such as water concentration, base catalyst concentration, and silane concentration were studied to control the final particle size. Scanning electron microscopy (SEM), transmission electron microscopy (TEM), and diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) were used for particle characterization. Their fluorescent properties were also investigated. In this presentation we will discuss the synthetic procedure, unique fluorescent properties, and potential applications of these fluorescent C-dots/silica particles.
The reaction of o-phthalaldehyde (OPA), a thiol compound, and a primary amine at alkaline pH to form the fluorescent isoindole derivative is one of the most important analytical approaches for the determination of amino acids and selected pharmaceuticals. The most common thiols used are the liquids mercaptoethanol and mercaptopropionic acid, both of which have offensive odors. We have considered alternative nonvolatile solid thiol compounds such as mercaptosalicylate, mercaptosuccinate, and captopril. Using lysine as the test amine, the aromatic sulfhydryl compound does not seem to react to form a fluorescent derivative and the succinate compound shows only a modest linear response. However, upon reaction of HPLC grade captopril with OPA and lysine, a strong stable fluorescent signal can be measured which appears to be comparable to that when mercaptopropionate is used. We plan to compare the reactivity of a monoamine (glycine), a diamine (lysine), and a hexamine (neomycin) with OPA and captopril. Linearity of response and detection limits will be compared. In addition, an indirect fluorescent method involving captopril has been developed to determine nitrite. The first reaction occurs between nitrite and captopril at acidic pH to generate nitrosocaptopril. This small volume solution is added to the borate buffered OPA and amine solution, and the resulting mixture will produce a fluorescent signal if captopril remains from the first reaction. Some experimental parameters, such as pH, temperature, reactant ratio and salt concentration have been studied indicating the method is sensitive and precise.

Keywords: Amino Acids, Fluorescence, Pharmaceutical
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
A Group of uniform materials based on organic salts (GUMBOS), i.e. organic salts with M.P between 25-250 oC was recently introduced by Warner [1]. It exhibit similar outstanding properties as ionic liquids except these materials are solids rather than liquids and, are being used for wide scope of applications [1]. In the present work, novel carbazole-based GUMBOS are synthesized and characterized. By changing the substituted group at carbazole, GUMBOS with tunable emission spectra are obtained. Detailed photodynamics study of these materials indicate that emission emanates from multiple excited states. In order to understand these complex emission process, lifetime measurements, excitation emission matrix and transient absorption spectroscopy are performed and the results obtained from these measurements are consistent and in well agreement. Furthermore, carbazole-based GUMBOS are highly photostable, displayed improved thermal stability, and exhibited high quantum yields. The unique photodynamic characteristics of these GUMBOS suggest the potential use of these materials in optoelectronics such as organic light emitting diodes (OLEDS) and as laser dyes.

Acknowledgement: Authors acknowledges support by the National Science Foundation (NSF) under Grant No. CHE-1307611.


Keywords: Fluorescence, Fuels\Energy\Petrochemical, Spectroscopy, Ultra Fast Spectroscopy
Application Code: Other
Methodology Code: Fluorescence/Luminescence
Semiconductor quantum dots (QDs) have received much acclaim for their bright, spectrally narrow, and tunable emission. A sometimes unappreciated advantage of QDs is their surface area, which provides new opportunities for bioanalysis. This presentation will describe some of our recent and ongoing efforts to develop fluorescent probes that exploit both the optical properties of QDs and their interface. These efforts include the design and characterization of so-called concentric Förster resonance energy transfer (cFRET) probes that utilize energy transfer networks to achieve multiplexed detection at discrete nanoparticle probes. For example, a configuration that combines peptide and aptamer probes with three cFRET pathways between a central QD and two fluorescent dyes is able to detect and distinguish between the concentration and hydrolytic activity of thrombin, a model protease. This capability is important because both concentration and activity are important in the biological function and regulation of proteases. Preliminary results on the combination of a QD with three fluorescent dyes, comprising six possible cFRET pathways toward triply multiplexed detection of protease activity, will also be presented. In addition to permitting the assembly of multiplexed FRET networks, the physicochemical properties of the QD interface--its ligand coating and peptide density in particular--can affect proteolytic activity with ramifications for the sensitivity and selectivity of activity-based assays. Finally, new results related to the design and function of self-contained QD-probes for the FRET-based detection of target nucleic acid sequences will be presented. Overall, this collection of research shows that the greatest value can be obtained from QDs when they are used as more than a substitute for fluorescent dyes, and one takes full advantage of the QD interface as a platform for bioanalysis.
LIMS validation is widely considered to be as expensive and time consuming as configuration and implementation. In this session, the presenter will talk about the lessons learned from multiple projects and best practices to reduce the validation timeline.

**Abstract Text**

LIMS validation is widely considered to be as expensive and time consuming as configuration and implementation. In this session, the presenter will talk about the lessons learned from multiple projects and best practices to reduce the validation timeline.

**Keywords:** Informatics, Laboratory, Laboratory Informatics

**Application Code:** Validation

**Methodology Code:** Laboratory Informatics
There is much more to validating your laboratory instrument and/or information systems than just running the vendor supplied Installation Qualification (IQ) and Operational Qualification (OQ) testing packages. In order to avoid costly bottlenecks in your validation effort, it is critical that you perform an accurate GAMP5 risk based assessment, undertake adequate validation planning, develop your deliverables (URS, FRS, Config Spec, etc...), and establish your validation process roadmap. Your effort can also become constrained if you are unable to secure the right resources to execute the validation activities.

Doing things right, the first time, is always our goal when beginning a project. However, over 60 percent of implementation projects fail to reach a go-live status, stay within budget, or meet the deadline; and validation if often the cause of the constraint. There are many factors that can cause such roadblocks, but there are also ways to avoid these detours. In the presentation, the author will share “Lessons Learned’ when validating laboratory instruments and information systems so you may increase your chances of a successful implementation.

Keywords: Computers, Laboratory Informatics, Software, Validation
Application Code: Validation
Methodology Code: Laboratory Informatics
In collaboration with the Royal Society of Chemistry, a project to develop the Chemical Analysis Metadata Platform (ChAMP) has been initiated to identify analytical science metadata that characterize chemical analysis methodologies. In this work, we propose that the development of standards for organization, representation, and annotation of analytical science information, be based around the definition of metadata in ChAMP. This project involves development of specifications for metadata collection, a taxonomy (organizational structure), ontologies to link the metadata semantically, design rules, and use cases.

This presentation will report the current state of ChAMP, show examples of application, and a discussion future developments.

Keywords: Informatics, Laboratory Informatics
Application Code: Other
Methodology Code: Laboratory Informatics
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Session Title: **Laboratory Informatics**

Abstract Title: **Ensuring that your Informatics Solution is Embraced by your Organization**

Primary Author: Dan Freel
CSols, Inc

Co-Authors:

Abstract Text

User acceptance is probably the most critical factor in a successful implementation of any laboratory informatics solution (e.g. LIMS, ELN, CDS). Unfortunately, many times the end user is overlooked and underutilized in the planning and implementation of these solutions. Though end users are often tasked with participating in the requirements gathering portion of the project, this crucial resource is often left out of the development and implementation process until final training just prior to roll-out.

In this presentation, strategies to best utilize existing resources in all phases of a laboratory informatics solution will be covered. Case studies will be introduced to provide strategies used for success. Specific project tasks will be outlined and processes to implement identified. Common pitfalls and avoidable errors will also be exposed. In addition, the presentation will look at the actual return on investment for both effective and non-effective scenarios. Beyond just efficiency in the laboratory, discussion will identify how wise use of existing resources will directly translate into meeting customer and regulatory requirements, audit readiness, personal development of employees, and the overall health of the organization.

Keywords: Laboratory Informatics, LIMS, Quality, Sample & Data Management

Application Code: Laboratory Management

Methodology Code: Laboratory Informatics
Laboratory Informatics Environments – Why Unified Platforms and Integration Now

R&D organizations IT groups are under pressure to evolve laboratory informatics environments and, not just support but also, to increase their organizational value. Therefore strategic themes for simplification have aimed at reduction and replacement of disparate software technologies such as CDS, ELN, LES, LIMS, SDMS, and data warehouses, with goals of consolidation, unification, and integration. This is leading to the emergence of hybrid informatics platforms for unified laboratory intelligence that can homogenize structured and unstructured data, while supporting the codification of knowledge to drive automated and intelligent workflows.

Having appropriate knowledge for effective decision-making in each R&D domain requires accessible analytical information. This drives the development of informatics architecture and components from desktop applications and virtualized environments, to enterprise databasing, thin/web/mobile clients and range of cloud-based deployment and access approaches.

This presentation will offer a view on emerging informatics strategies, integration and platforms with an emphasis on the applicability of technologies in the Unified Laboratory Intelligence (ULI) category for chemistry R&D subdisciplines that feature a substantial variety and volume of analytical data in their workflows.

Keywords: Laboratory Informatics, Software
Application Code: General Interest
Methodology Code: Laboratory Informatics
President Obama signed the Leahy-Smith America Invents Act (AIA) into law in September of 2011. This affected many sections of the existing U.S. patent statutes, the most talked about being the shift from a first-to-invent patent system to a first-to-file system. The first-to-file patent system went into effect in March of 2013.

In the “old days” our patent system granted the patent to an invention based on who could prove they came up with the idea first. This is the “first-to-invent” patent world. To prove who was first you needed “business records” which are court admissible records that are dated, signed, witnessed records that you could prove have not been changed or tampered with. Business records could be electronic and an Electronic Laboratory Notebook (ELN) was the perfect system to capture and store them. ELNs provided date/time stamping, electronic signatures, and audit trails. Intellectual property preserved! ELN justified and paid for!

Now our patent laws have changed. Now, the first person to file the patent or disclosure is the one granted the patent. This is the “first-to-file” patent world. So the need for business records goes away! Uh oh! Do you still need ELNs? The quick answer to this question is yes!

This presentation will summarize the changing information landscape that ELNs address as well as the technical, business, and legal reasons why ELNs are still needed in the organization.

Abstract Text

| Keywords: | Informatics, Laboratory Informatics, Scientific Data Management, Software |
| Application Code: | Other |
| Methodology Code: | Laboratory Informatics |
Instrumentino is an open-source modular graphical user interface framework for controlling Arduino based experimental instruments. It expands the control capability of Arduino by allowing instruments builders to easily create a custom user interface program running on an attached personal computer. It enables the definition of operation sequences and their automated running without user intervention. Acquired experimental data and a usage log are automatically saved on the computer for further processing. The use of the programming language Python also allows easy extension. Complex devices, which are difficult to control using an Arduino, may be integrated as well by incorporating third party application programming interfaces into the Instrumentino framework.

In this presentation, the Instrumentino [1] infrastructure will be explained and the benefits of its usage will be discussed. A series of example systems in the field of analytical chemistry, already using Instrumentino, will be presented as well.

The main goal is to expose the Instrumentino framework to fellow researchers and promote future collaborations.

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<td>Primary Author</td>
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**Abstract Text**

Lab managers and scientists struggle every day with efficiently managing large and small laboratories containing instruments from multiple vendors. Making the right decisions concerning how laboratory assets are deployed, utilized and managed throughout their lifecycle allows labs to make the proper choices and implement changes that will increase productivity, gain efficiencies and drive overall business improvement. However, collecting and compiling the data required to drive those decisions can be challenging.

This presentation explores the impact of mobile applications and visualizing instrument performance using new techniques with the objective of creating a more interactive and informative lab environment. These powerful reporting tools to provide you with a visual and interactive way to review laboratory asset data as well as exposing instrument “hot spots” where attention needs to be invested. Additional possibilities include visualization of system performance over time to more precisely see when service is required. Gaining higher efficiencies in the lab results in higher levels of uptime and instrument availability as well as better overall laboratory operation and saved costs.

**Keywords:** Data Analysis, Database, Lab Management, Laboratory Informatics

**Application Code:** Laboratory Management

**Methodology Code:** Laboratory Informatics
Phosphorylated carbohydrates play an important role in energy utilization. Specifically, the phosphorylated disaccharide trehalose-6-phosphate (T6P) is essential for, among other functions, carbon regulation in plants. Resolving and quantifying phosphorylated sugars, however, can be difficult due to the interaction of the phosphate group with stainless steel components of the HPLC system, resulting in a tailing effect that can impair the sensitivity of the method. Additionally, many HILIC methods use high concentrations of mobile phase modifiers and pH > 11 to separate T6P from other phosphorylated disaccharides (sucrose-6-phosphate, S6P), pushing the limits of column pH recommendations. Herein, we report the benefits of incorporating the reverse-phase “tail-sweeping” reagent, methylphosphonic acid (Han et al. 2013, Anal. Chem.), in the aqueous mobile phase along with minimal amounts of triethylamine (TEA) in the organic mobile phase to maintain alkaline conditions necessary for HILIC resolution of T6P and S6P. Addition of methylphosphonic acid significantly reduced peak tailing, producing a 30% increase in peak height while maintaining baseline resolution of T6P and S6P. Sensitivity was further improved by minimizing the amount of TEA without adversely affecting the separation in both standards and unspiked plant extracts. The proposed method also resolves and thereby enables simultaneous quantification of the phosphorylated monosaccharides of glucose and fructose such as G6P, G1P, F6P, and F1P. The method was then applied to flooding tolerant and intolerant rice varieties to determine the effect of submergence stress on T6P and other phosphorylated sugars to further elucidate plant stress response.

Keywords: Bioanalytical, HPLC, Mass Spectrometry, Metabolomics
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Pteridines are a diverse family of endogenous metabolites that may serve as useful diagnostic biomarkers for disease. While many preparative and analytical techniques have been described for limited pteridine analysis in biological fluids, comprehensive pteridine profiling and intracellular pteridine detection to support biomarker qualification efforts have continued to face analytical challenges. In this study, a simple, rapid, specific, and sensitive high performance liquid chromatography – quadrupole time-of-flight – mass spectrometry (HPLC-QTOF-MS) method was developed to simultaneously quantify seven intracellular pteridines and semi-quantitatively monitor 17 additional intracellular pteridines. In addition, pteridines were extracted from the cell lysate using a newly developed and optimized pteridine extraction method. The resulting method was validated through evaluation of spiked recoveries (84.5% - 109.4%), reproducibility (2.1% – 5.4% RSD), method detection limits (0.1 – 3.0 µg/L) and limits of quantitation (0.1 – 1 µg/L), and application in two cell lines including healthy embryonic kidney HEK-293A cells and non-small cell lung cancer A549 cells. This new platform enables comprehensive pteridine analyses to generate novel pteridine profiles for various pathological conditions. The detailed HPLC-QTOF-MS method, extraction protocol, and results will be presented at the conference.

This study was supported by the US National Science Foundation and the National Science Foundation of China.

Keywords: Bioanalytical, Liquid Chromatography/Mass Spectroscopy, Metabolomics, Metabonomics
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
The study presents a novel protocol for accurate quantification of reduced (GSH), oxidized (GSSG) and total (tGSH) glutathione in biological samples using molecular speciated isotope dilution mass spectrometry (EPA Method 6800), which involves isotopic ratios and not calibration curves. For GSH and GSSG measurement, the sample was spiked with isotopically enriched analogues of the analytes, along with N-ethylmaleimide (NEM), and treated with acetonitrile to solubilize the species via protein separation. After centrifugation, the supernatant was analyzed by hydrophilic interaction liquid chromatography–tandem mass spectrometry (LC-MS/MS), and the GSH and GSSG were quantified with simultaneous tracking and correction for auto-oxidation of GSH. For tGSH measurement, the sample was spiked with isotopically enriched GSH and treated with dithiothreitol to convert the disulfide-bonded glutathione to GSH. After removing the protein, the supernatant was analyzed by LC-MS/MS and the analyte was quantified by single-spiking isotope dilution mass spectrometry (IDMS). Red blood cell (RBC), whole blood and saliva samples collected from healthy subjects were analyzed. The protocol was validated using spike recovery tests and by analyzing synthetic standard solutions. The concentrations of tGSH in the RBC and whole blood samples were two orders of magnitude higher than those found in saliva. The fractions of GSSG were 0.2–2.2% (RBC and blood) and 15–47% (saliva) of the free glutathione (GSH + 2xGSSG) in the corresponding samples. Up to 3% GSH was auto-oxidized to GSSH during sample workup; the highest oxidations (>1%) were in the saliva samples.
Febuxostat is a novel non-purine selective inhibitor of xanthine oxidase (XO), which is used for the treatment of gout. In the present study, a rapid and sensitive liquid chromatography-tandem mass spectrometry (LC–MS/MS) method for simultaneous determination of febuxostat and its three active metabolites, hydroxylated febuxostat 67M-1 and 67M-2, carboxylic acid 67M-4 in human plasma has been developed and validated for the first time. After addition of internal standard (IS) losartan, plasma samples were pre-treated by one-step protein precipitation with methonal. Febuxostat, three metabolites and the IS were separated on a ZORBAX SB-C18 column (50mm×4.6mm, 5µm) using gradient elution with a mobile phase consisting of acetonitrile, water and formic acid. Detection was performed on a triple quadrupole tandem mass spectrometer by selective reaction monitoring (SRM) mode to monitor the precursor-to-product ion transitions of m/z 317 → 261 for febuxostat, m/z 333 → 261 for 67M-1 and 67M-2, m/z 347 → 261 for 67M-4 and m/z 423 → 207 for IS respectively using a positive electrospray ionization (ESI) interface. The total run time was about 12 min per sample. The method was validated over the concentration range of 10–20,000 ng/mL for febuxostat, 1.0–270 ng/mL for 67M-1 and 67M-2, and 0.8–250 ng/mL for 67M-4 respectively. The extraction recoveries of all the analytes at three concentration levels were consistent. The intra-day and inter-day precisions of the investigated components exhibited an RSD within 14.7%, and the relative errors ranged from 4.3% to 5.1%. The proposed method has been successfully applied to a clinical pharmacokinetic study of febuxostat in Chinese volunteers after oral administration of a single dose of febuxostat at 40, 80 and 120 mg.

Abstract Text

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Keywords: Bioanalytical, Liquid Chromatography/Mass Spectroscopy
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Development of SPME–LC-MS Method for Concomitant Measurement of Rocuronium Bromide and Tranexamic Acid in Plasma: Application to Pharmacokinetic Study

A high-throughput method using solid-phase microextraction coupled to liquid chromatography-tandem mass spectrometry (SPME-LC-MS/MS) for concomitant determination of tranexamic acid and rocuronium in human plasma was developed and validated. Because of instability of rocuronium in collected plasma samples the standard analytical approaches employ acidification of the sample, what affects the binding equilibrium of the drug and consequently no information on the free/bound concentration can be obtained. Contrary to these protocols, the proposed method requires minimum sample handling and no ion pairing and derivatization procedure. A weak cation exchanger coating was chosen for the first time as the best extracting phase for selected drugs, guaranteed a good recovery, minimum carry-over and selective extraction. SPME procedure met all Food and Drug Administration acceptance criteria for bioanalytical assays at three concentration levels, for both selected drugs. Post-extraction addition experiments showed that matrix effects were less than ±3%. Therefore, a weak cation exchange thin-film solid phase microextraction (WCX TF-SPME) approach presented here offers simple cleanup procedure and full quantitation of the drugs in plasma as one the most challenging matrices with regards to its complexity. In addition, 96-well plate format of WCX TF-SPME system provides many advantages like high throughput analysis for up to 96 samples in 30 min (19 s/sample), a need of a small amount of plasma sample (0.8 mL), and simple sample preparation protocol, which shows a promise for possible on-site application in hospital e.g. to support of clinical pharmacokinetics studies.

Keywords: Biological Samples, Clinical/Toxicology, Liquid Chromatography/Mass Spectroscopy, Sample Preparation
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Currently available biomarkers such as anionic sugars lack sensitivity for early detection of health related diseases, in particular certain cancers. To address this challenge, paired ion electrospray ionization (PIESI) was employed for ultra-sensitive detection of anionic sugars (i.e., sugar phosphates, carboxylic sugars and sialic acids). The PIESI-MS approach provides enhanced sensitivity for these anionic sugars, and overcomes the drawbacks of the less sensitive negative ion mode ESI-MS by detecting the anionic sugars in the positive ion mode at higher m/z where the background noise is less. Using PIESI-MS, the absolute LODs for these tested biomarkers were one to two orders of magnitude better compared to negative ion mode detection. Up to three orders of magnitude improvement were obtained compared to other reported HPLC-MS based methods performed in the negative ion mode. Structurally similar anionic sugars such as isobaric sugar phosphates were chromatographically separated and detected by HPLC coupled with PIESI-MS. The developed HPLC-PIESI-MS method may provide a powerful approach for the sensitive detection of disease biomarkers and is useful for diagnosis.

Keywords: Bioanalytical, Liquid Chromatography/Mass Spectroscopy, Metabolomics, Metabonomics, Separation
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Collection of dried blood spots is a minimally invasive technique to sample blood on cards of filter paper for biochemical or genetic analysis. This technology offers the advantage of being much less invasive than conventional venous blood sampling, and requires only limited infrastructure for sampling and logistics. In practice, a sampling can be achieved anywhere and without specialist training. Such methodology is of particular interest for application in web-based studies, in which the participants are remote at home. The Food4Me study is a large multi-center, web-based personalized nutrition intervention, which aims to compare the effectiveness of three levels of personalized nutrition (based on dietary, phenotypic and genotypic data) on behavioral changes (diet and physical activity) and health outcomes (blood metabolites and obesity-related anthropometrics), www.food4me.org. In total, 4123 dried blood spot samples from participants recruited by seven clinical centers in different European countries have been analyzed for 25-hydroxy vitamin D3 (25-OH-D3). Three time points, baseline, 3m, 6m, have been analyzed from each participant who finished the study. Analysis was performed by LC-MS/MS with APPI. Blood with known, endogenous 25-OH-D3 content was used for calibration. The current method was developed specifically for the FOOD4Me study, and was validated following EMA guidelines. The results gave valuable information on the acceptance and possible issues associated with unsupervised sampling by the participants at home, especially with regard to sample quality. For each center a status distribution for men and women was compiled. The data was further investigated regarding variations between time points of the same subject, possibly reflecting influence of seasons, sun exposure and/or food/supplement intake data.

Funding statement: This project is supported by the European Commission, grant number 265494.

Keywords: Bioanalytical, Biological Samples, Liquid Chromatography/Mass Spectroscopy, Method Development
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Adenosine 5’-triphosphate (ATP), adenosine 5’-diphosphate (ADP) and adenosine 5’-monophosphate (AMP) play critical roles in cellular metabolism as well as energy transfer and storage. When cells are exposed to different types of bioactive materials, the normal cellular functions can be affected and the concentrations of ATP, ADP and AMP may be significantly changed. Therefore, the measurement of these molecules is of importance to evaluate the cellular energy changes upon exposure to biomaterials. In this study, a simple and sensitive ultra-fast ion-exchange chromatography coupled with tandem mass spectrometry (UFIC-MS/MS) method was developed to determine ATP, ADP and AMP in biological samples. Three biomolecules can be fully separated and detected by MS/MS to the low µg/L level. This method has been applied to analyze the ATP, ADP, and AMP levels in cell culture media released from human fibroblast cell line CCL-110 upon exposure to bioactive glass nanofibers, which are used in soft tissue engineering. The detail experimental designs, results, and discussions will be presented at the conference. This research was supported by the Center for biomedical Science and Engineering (CBSE) and department of chemistry in Missouri University of Science and Technology.
## Abstract Text

The detection of pharmaceuticals and personal care products (PPCPs) and pesticides in surface waters is critical for their combined environmental and health effects. Some of these compounds are classified as carcinogenic, endocrine disrupting, and they may act synergistically to cause these effects. Solid phase extraction (SPE) using disks or cartridges is a commonly used method for extracting these environmental contaminants, but the efficiency of these two methods has not been thoroughly compared to determine which is the optimum choice for these compounds and their metabolites. Additionally, liquid-liquid extraction is used far less frequently in the extraction of PPCPs and pesticides from surface water and hasn’t been rigorously compared to SPE methods. In this study, we comprised a list of 12 commonly found PPCPs and 33 pesticides and compared these three extraction methods using liquid chromatography-time of flight mass spectrometry (LC-TOF-MS) and gas chromatography-mass spectrometry (GC-MS) as our detection systems. Our results showed that SPE disks gave a significantly higher recovery than the cartridges for both groups of compounds, while liquid-liquid extractions had higher precision and recoveries than SPE disks for most of the compounds and their metabolites that were tested. We conclude that, although SPE disks have the advantage of higher throughput, liquid-liquid extraction is an excellent choice when preparing samples containing trace PPCPs and pesticides, and should be used for their combined analysis in surface and wastewater samples.

### Keywords:
- Environmental/Water
- Extraction
- Pharmaceutical

### Application Code:
- Environmental

### Methodology Code:
- Liquid Chromatography/Mass Spectrometry
### Confirmation of Haloacetic Acids in Bulk Sodium Hypochlorite Solutions Used for Drinking Water Disinfection

Chlorination is the most commonly practiced method for water disinfection. An increasing number of water utilities are turning to the use of bulk hypochlorite solutions due to homeland security concerns. It is well known that chlorination produces disinfection by-products (DBPs) such as trihalomethanes (THMs) and haloacetic acids (HAAs). These DBPs are carcinogenic and the United States Environmental Protection Agency has established maximum contaminate levels for Total THMs and Total HAA5 to be 0.080 mg/L and 0.060 mg/L, respectively. The majority of studies have focused on the formation of DBPs in the distribution system while much less is known about their presence in bulk hypochlorite solutions.

Recently, significant concentrations of three regulated HAAs were detected in thirty bulk hypochlorite solutions from twenty-four water utilities. These measurements were made by post column reaction-ion chromatography where the HAAs are separated by anion-exchange chromatography followed by a post-column reaction to produce a fluorescent product. The HAA concentrations in bulk hypochlorite solutions could contribute to HAA concentrations in the distribution system through dilution.

An alternative method employing liquid chromatography-tandem mass spectrometry (LC-MS/MS) will be presented for the detection of HAAs in bulk hypochlorite solution. The optimization of method parameters, MDL, accuracy, and precision of the method will be discussed. Finally, analysis of real world bulk hypochlorite solutions will be presented.

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**Keywords:** Environmental Analysis, Liquid Chromatography/Mass Spectroscopy, Method Development, Water

**Application Code:** Environmental

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
The objective of this research is to develop a target based extraction method that is capable of detecting a variety of chemotherapeutic agents at a level that is appropriate for the analysis of drinking, surface, and wastewater. Identification and quantification of chemotherapeutics throughout water purification processes have not been reported in the United States. There currently is not an established method for targeting a variety of chemotherapeutic drugs in the initial and final stages of the wastewater treatment process. Although these pharmaceuticals share a common physiological effect, their structures and chemistries vary dramatically. Developing an extraction method that will retain trace amounts of these diverse compounds proves challenging and places significant demands on both the sample preparation steps and instrumental analysis.

The identification of samples will be conducted using an UHPLC-triple quadrupole mass spectrometer to yield the necessary overall sensitivity to detect trace level contaminants. A method for the analysis using a UHPLC-triple quadrupole mass spectrometer was optimized and provides sufficient separation of a variety of chemotherapy standards. The stationary phase column chemistries that provide the most efficient separation of a mixture of chemotherapeutic agents were determined using UHPLC-PDA. The final step is to optimize a method for extraction of a variety of chemotherapeutic agents from surface, drinking, and wastewater. This involves investigating solid phase extraction phases in order to maximize percent recovery of the targeted compounds. Due to the compounds trace levels a large-volume SPE-based sample preparation technique that is compatible with UHPLC analyses will be developed.

Abstract Text

Keywords: HPLC, Mass Spectrometry, Solid Phase Extraction
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
Iron (Fe) and manganese (Mn) have been associated with discoloration of drinking water in the Northwest UK. In the cases of discoloration, iron network pipework has been replaced with plastic. This should have reduced the number of discoloration events if iron was the sole cause of the problem. Unfortunately, this has not reduced the number of failures. Both Fe and Mn have also been associated with toxicity at high levels particularly with reference to neurotoxicity, which has been discussed by Elsner and Spangler (1).

The aim of this study was to analyse iron and manganese species in drinking water. Water samples were collected in the Northwest region of the UK to assess discoloration issues. The samples were analysed using Thermo X series ICP-MS with a Thermo 4000 series HPLC being used for speciation and sample introduction.

This worked considered the use of ion chromatography separation of Fe(II), Fe(III) and Mn(II). In reverse phase mode, Thermo Accucore columns were employed with an EDTA and TBAH (tetrabutyl ammonium hydroxide) mobile phase. This gave rapid analysis times for Mn(II) i.e. less than 5 minutes. Cationic exchange material (Dionex CS5) in conjunction with a PDCA (pyridine dicarboxylic acid) was investigated to separate Fe(II) and Fe(III). Data will be presented of the separation and will show how the speciation obtained can be used to identify the source of discoloration in the drinking water samples.

A spiked recovery study was carried out and values between 95-105% were obtained. The results indicated that discoloration may not be due to iron but to manganese within the samples and also the water treatment process. The implication is that discoloration maybe minimized without the need for costly pipe replacement work.


Keywords: Environmental/Water, HPLC, ICP-MS, Metals
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
A myriad of pharmaceuticals have been detected as contaminants in surface and wastewaters all around the globe, which can be linked to adverse health effects of local residents in those areas. According to the Center for Disease Control, eastern North Carolina has the highest accounts of stroke, heart disease, diabetes, and other diseases than any other region in the state. In this study, multiple water samples were obtained along various points of the Tar River and various stages of water treatment plants in eastern North Carolina to determine the types and levels of pharmaceuticals and personal care products (PPCPs) in surface waters and their potential link to the high incidences of disease in this area. Samples were prepared by solid phase extraction (SPE) or liquid-liquid extraction and analyzed for parent pharmaceutical compounds and their metabolites by liquid chromatography-time of flight mass spectrometry (LC-TOF-MS) and gas chromatography-mass spectrometry (GC-MS). Many parent PPCPs and their metabolites were detected in municipally treated water as well as in the Tar River including: carbamazepine, iminostillbene, oxcarbazepine, epiandrosterone, loratidine, gabapentin, estradiol, triclosan, and others. From these findings, we conclude that PPCPs are currently water contaminants in eastern North Carolina, and may play a role in the frequency of differing disease states while also having a potential environmental impact on aquatic biota in this region.

Funding for this project was provided by an internal grant from the ECU Division of Research and Graduate Studies.

Keywords: Biopharmaceutical, Environmental/Water, Liquid Chromatography/Mass Spectroscopy, Toxicology
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
During the development of proteins or monoclonal antibodies as therapeutics, it is critical to monitor the deamidation of asparagine residues as part of the stability study. LC-MS tryptic peptide mapping has become the method of choice for the analysis of deamidated degradation products, typically requiring 1.5 hours or even longer to achieve sufficient chromatographic separation between the native and the deamidated species. Subsequent relative quantitation by LC/MS peak area integration based on accurate mass XIC has been considered the gold standard, widely accepted, and routinely used in both industry and academia.

In this presentation, we will demonstrate an important pitfall and a systematic error in accurate mass XIC quantitation, due to a confluence of factors including aggressive settings on mass tolerance window, the mass spectral overlap between the A+1 isotope of the native peptide and the monoisotope peak (A) of the deamidated peptide, and the MS centroiding process. A novel and unique peaks shape calibration and analysis approach based on full profile mode data without centroiding will be presented as a fast and accurate alternative to LC peak area quantitation, allowing for highly accurate mixture analysis and the relative quantitation of deamidated and native peptides. Through comprehensive investigation on 9 different peptides with actual experimental data acquired with Orbitrap MS, we conclude that quantitation by mass spectral accuracy is at least as accurate as that from conventional LC peak area integration when there is true baseline separation. Equally accurate results can be obtained even with limited LC separation or no separation at all, i.e., through direct infusion. Interestingly, this approach also allows for the quantitative analysis of other degradation products such as multiply deamidated peptides or tryptophan oxidation products, all simultaneously in an one step full mass spectral calibration and analysis process.

Keywords: Biopharmaceutical, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
It was desired to administer crushed tablets in various solutions and food matrices. To demonstrate stability under the conditions of use, appropriate analytical methodology is required to quantify the potency and potential degradants for each proposed sample matrix. This presentation will describe the challenges encountered during method development concerning sample matrix interference for key degradants, sample extraction robustness, and the mitigation strategy. Specifically, the formation of an early-eluting API degradant could occur in the presence of two matrices and significant matrix interference is observed with detection and quantification of the degradant using conventional techniques. Additionally, the interference varied as a result of the composition of the two food vehicles, apple juice and applesauce. Data presented demonstrate that analysis by LC/MS ensures accurate quantification of the potential degradant in all desired vehicles: water, 5% Dextrose in water, apple juice and applesauce. Additionally, sample preparation techniques were required to mimic clinical administration techniques. Data indicates that a single tablet crush and rinse preparation satisfies the clinical requirement while demonstrating that recovery and quantification risks are minimized.
Robust, reliable chromatographic methods must be based on complete separation of all sample components. For reversed-phase methods, it has become common practice to screen organic solvents, column chemistries, and gradient slope as the first steps in developing a separation method. It is, however, also recognized that adjustment of mobile phase pH can also provide important and useful changes in selectivity where the analytes are ionizable. This manipulation has often been underutilized in method development because the preparation of buffered mobile phases is time consuming and labor-intensive. Most often, only widely separated pH conditions are tested to maximize retention. For groups of related compounds, however, small changes in pH can be more effective because the analytes behave as though partially charged. We have developed techniques and software to facilitate the preparation of buffered mobile phases from concentrated stocks using the solvent proportioning capabilities of the UPLC pump. To extend the utility of this approach, a buffer system has been developed that covers the pH range from 2-12 with good linearity and capacity. To facilitate peak identification and tracking, the buffers are volatile and transparent. Electrospray MS detection can, therefore, be combined with UV spectral detection using a photodiode array detector. We have shown the use of this buffer system for developing separations of pharmaceutical formulations as well as a variety of industrial chemicals. The fully automated preparation of mobile phases with different pH properties helps to ensure the optimum selectivity for separation of analytes in reversed-phase liquid chromatography.
In recent years, it has become obvious that analytical chromatographic methodology is greatly improved when the stationary phase uses core-shell media as compared to fully porous media. The core-shell media will provide higher efficiency, improved sensitivity, and increased resolution when compared to what could be achieved with the same stationary phase on the same size fully porous media. Core-shell stationary phases are available in 5um particle size for prep chromatography and all of the positive chromatographic properties observed at the analytical scale translate to the prep scale. However, the goals for prep methodologies are different than for analytical methods. The prep chromatographer is looking to collect material and optimize the triangle of purity, yield and throughput for that particular project. The properties achieved by core-shell material will help significantly with the purity and yield aspect but throughput is very dependent upon loading before the purity and yield are impacted.

The conventional thinking about loading is that it is driven by the surface area of the stationary phase media. Since core-shell media is not porous in the center of each particle, it has less surface area than an equivalent sized particle that is fully porous. The work presented here evaluates how significant this difference in surface area is with respect to loading for prep chromatography. Loading studies on both core-shell and fully porous media for several pharmaceutically related compounds will be presented.

Keywords: Extraction, Liquid Chromatography, Pharmaceutical, Supercritical Fluid Chromatography
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Transfer of established HPLC methods across both HPLC and UHPLC chromatographic instrumentation from different vendors requires careful consideration of chromatographic principles as well as an understanding of each instrument's operating parameters. For example, the instrument's attributes, including temperature control and fluidic pathway, can all impact the fidelity of the separation and, thereby, the overall success of method transfers. Instrument parameters, such as operating pressures and flow rates, as well as method conditions, can also limit the transferability of methods to/from instruments. To overcome these challenges, all of the factors must be considered and accounted for when transferring an HPLC method from instrument to instrument and across different vendors.

We will present a series of studies illustrating methods transfer of HPLC methods across multiple instruments and instrument vendors. Chromatographic properties such as gradient delay, sample considerations (sample solubility and diluent) and temperature will be assessed and factored into the method transfer studies. These studies will also evaluate the impact of instrument attributes including temperature control, system volume, injector design and pump characteristics. Transferability of the method will be assessed through system suitability criteria (retention time, relative retention time, area, etc.). Consideration is made to conduct method transfer in accordance with USP-NF Chapter 621 guidelines for allowable adjustments to compendial methods. The studies will culminate in guidelines for successful methods transfer of HPLC methods across both UPLC and HPLC instrumentation.

Keywords: HPLC, HPLC Columns, HPLC Detection, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
New stationary phases consisting of high performance R,S-hydroxypropyl ether derivatized cyclodextrin (HP-RSP CD) and native cyclofructan-6 (CF-6) bonded to silica superficially porous particles (SPP) were developed and analyzed in hydrophilic interaction chromatography (HILIC) mode. These new SPP stationary phases (2.7 μm diameter) were evaluated and compared to HP-RSP CD and CF-6 bonded to fully porous particles (FPP) of 5 μm and 3 μm diameters. The HP-RSP CD and CF-6 SPP phases showed faster and more efficient chromatography compared to both 5 μm and 3 μm in diameter FPP phases. The analysis of SPP phases yielded a reduction of analysis time by 50 to 70% with a higher value of N/min over the entire range of flow rates analyzed compared to the FPP phases. Van Deemter plots of the SPP phases showed a higher optimum flow rate compared to the FPP phases. In summary, HP-RSP CD and CF-6 SPP HILIC phases showed clear advantages compared to the FPP counterparts in terms of high throughput and efficient separation of compounds.
Analytical chromatographic methodologies can use a wide range of eluent modifiers to optimize their chromatography. Prep Chromatography processes are more limited in the use of eluent modifiers. The only requirements for modifiers in analytical HPLC are compatibility with the detector, not precipitating the analyte and not causing degradation within the timescale of the chromatographic run. The requirements for modifiers used in Prep Chromatography must meet the same requirements but also must not impair the isolation of the final material. There can’t be any undesired salt formation, degradation during isolation, or hindering of the desired crystallization.

Trifluoroacetic acid (TFA) is a commonly used modifier for chromatography. TFA is used to improve the peak shape or change the column selectivity for the analytes of interest. One of the best attributes TFA has for use in Prep Chromatography is that it is volatile. Typically it can be removed by drying in an oven, under vacuum or with a rotovap. However, sometimes TFA will form a salt with the isolated compound and cannot be removed with the usual techniques. Another issue encountered with TFA, is that some compounds degrade in its presence and this degradation is often accelerated during the concentration / isolation process. The work presented here will describe methodology we have developed to remove TFA from collected Prep Chromatography fractions by using solid phase extraction. This technique has been applied at several different scales from mg to multigram with fraction volumes as large as several liters.

Keywords: Drug Discovery, Flavor/Essential Oil, Pharmaceutical, Prep Chromatography
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
The advantages of the analysis and purification of basic compounds by RP-LC in high-pH mobile phases have been clearly established in the past ten years since the introduction of alkaline resistant hybrid-type fully-porous sorbents. These proven benefits include extended retention for polar basic analytes, improved peak shapes, different selectivity as well as increased loading capacity. The advancements in core-shell particle technology of recent years also bring to reality the benefits of unparalleled resolving power for the demanding chromatographic applications. These particles enable the development of analytical methods with shorter run times and higher sensitivity. The purpose of this study is to demonstrate the benefits of a new stationary phase that is optimized for the challenging analysis and purification of basic compounds by combining the hybrid organo-silica grafting and core-shell technologies.

**Abstract Text**

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Superficially porous alkyl phases such as Poroshell EC-C18 are widely used for reversed-phase HPLC. Superficially Porous HPLC columns such as Poroshell 120 show comparable efficiency to sub 2 micron particles with about half the back pressure. Columns packed with these materials can be used in older HPLC instruments or in new higher pressure instruments for high resolution in longer column formats. However, analysts occasionally encounter difficult separations for which selectivity, ruggedness or reproducibility is not easily obtained with a C18 column. These separations might require the use of bonded phases or conditions that may yield a different selectivity. In these cases a method that is orthogonal, or offering differing relative retention to the first method, is desirable.

The use of pH is particularly powerful way to affect selectivity, especially with compounds of pharmaceutical interest. As these compounds are ionizable, pH can affect the retention time dramatically. With the introduction of newer more pH stable superficially porous particle columns such Poroshell HPH-C18, a wider variety of buffers is available for use by the analyst without sacrificing lifetime at high pH compared to silica based columns. In addition to pH, choices of bonding and end capping chemistry can vary selectivity. Retention factors are measured for neutral, acidic and basic compounds on a variety of superficially porous columns. Several chromatographic conditions are varied including buffer/pH modifier, organic modifier (MeOH or ACN), and column chemistry (C18, Polar Embedded, Phenyl-Hexyl, PFP, end-capped vs. non – end capped). These conditions are examined to determine selectivity differences and similarities across a range of commonly used LC/MS compatible, preparative and LC only compatible conditions. The most orthogonal separation conditions are identified.

Keywords: Amino Acids, HPLC, HPLC Columns, Liquid Chromatography/Mass Spectroscopy
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Aggregate analysis via gel filtration chromatography (GFC) is a primary quality indicating method for any protein, and a required lot release test for most protein therapeutics. As protein aggregates are typically the principal cause of drug neutralizing antibodies, determination of the level of aggregation in a sample is important in determining the safety of a drug. In an ideal setting GFC can deliver a highly accurate value of covalent and non-covalent protein aggregates in solution. Unfortunately, secondary interactions between the stationary phase in a GFC column and aggregated proteins in solution can adversely affect their quantitation leading to inaccurate results. Recent innovations in column chemistries by several leaders in the field have overcome some of these issues; however different operating conditions are often required to achieve accurate results. In addition, some new products feature smaller particle media which have allowed for rapid GFC separation that require further HPLC method development and different analytical challenges. In this study several new GFC column technologies were evaluated including some sub-2u columns recently introduced into the market. Secondary interactions were assessed over various salt, pH, and temperature conditions for the mobile phase. In several cases changing the ionic strength of the mobile phase could significantly increase the recovery of protein aggregate. In addition, repeat injections of proteins can “prime” a column by saturating sites of interaction leading to higher recovery of proteins. Results from these experiments attempts to present a methodology for making GFC methods that properly report the level of protein aggregation in therapeutic drug samples in a robust and reproducible manner.
Reversed phase high performance liquid chromatography (RP-HPLC), hydrophilic interaction liquid chromatography (HILIC) and ion exchange chromatography (IEC) technique were investigated for the simultaneous determination of heparin and its impurities including oversulfated chondroitin sulfate (OSCS) and dermatan sulfate (DS) in heparin products. Different types of HPLC columns, e.g. ODS, HILIC amide and strong anion exchange (SAX) columns were studied. Composition of mobile phases including types and pH of buffer and type and amounts of organic modifiers (methanol and acetonitrile) were varied. The examined RP-HPLC and HILIC conditions did not retained heparin and its impurities, resulting in overlapping peaks at the solvent front. SAX-HPLC (gradient elution of sodium chloride and Tris phosphate buffer pH 3.0) showed some retention of the analytes. Thus, IEC seemed to be the most suitable technique among the three selected techniques for the analysis of heparin and its impurities. Lowering mobile phase pH and addition of acetonitrile enhanced the separation (Rs = 1.46). The separation of heparin, DS and OSCS (Rs above 2.0) was achieved on a SAX column with a gradient elution of 10-70% of 2.5 M sodium chloride and 20 mM Tris phosphate buffer pH 2.1 at a flow rate of 0.6 ml/min and UV detection was at 215 nm. The method was fully validated according to the ICH recommendations and applied to analysis of raw materials and heparin products in Thailand. The method will be valuable to the Thai Food and Drug Administration (FDA) and hospitals for selection of heparin products.

Keywords: Liquid Chromatography, Method Development, Pharmaceutical, Validation

Application Code: Pharmaceutical

Methodology Code: Liquid Chromatography
Preparation and Characterization of Nanomaterials for Bioanalytical Applications

Metal Oxide Nanoparticle Based Paper Sensors for Field Analysis

The uses of nanoparticle based analytical technologies are dramatically increasing in the fields of analytical chemistry, nanotechnology, clinical diagnosis and the food industry. In this presentation, we describe development and characterization of portable nanoparticle based-assays, similar to a small sensor patch, for rapid and sensitive detection of target analytes and the use of these particles as colorimetric probes in bioanalysis. These assays are based on the immobilization of metal oxide nanoparticles onto filter paper, which change color after interaction with the target analyte by means of redox and surface chemistry reactions. Paper based sensors have advantages in terms of low-cost and portability, allowing development of point-of-care (PoC) diagnostic devices. Integration of metal oxide nanoparticles onto a paper platform enables realization of a sensor that does not require external reagents, as all the sensing components are fixed onto the paper platform. The proposed mechanism can find wide application as a general approach for simplifying detection schemes of colorimetric bioassays, e.g. enzyme, gene, immune and aptamer assays and related affinity sensing methods. Several examples of such applications will be presented.

Keywords: Agricultural, Bioanalytical, Biosensors, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Portable Instruments
## Abstract Text

Current bioanalytical sensing and imaging applications utilizing nanomaterials require distinct properties such as high brightness, chemical inertness and colloidal stability in physiological media. As many established nanoparticle systems either lack at least one of these properties or have several disadvantages (for example, complicated synthesis, tedious surface engineering), researchers continue to seek alternative materials. Recently, carbon dots (Cdots), have emerged as promising candidates for new applications, as they combine all of the desired properties mentioned above.

The reported Cdots, prepared via hydrothermal treatment of starch and tryptophan, are closely related to graphene quantum dots in terms of luminescence properties with the advantages of lower cytotoxicity and excellent colloidal stability. The optical properties, as well as possible toxic effects were characterized in detail. These water-dispersible particles with a diameter of 1.6 ± 0.8 nm exhibit unique luminescence properties which enable multicolor imaging of mammalian cells. Extreme photostability as well as high quantum yields of up to 26% render them as attractive probes in confocal laser scanning microscopy (CLSM). A concentration dependent wavelength shift in the fluorescence of the Cdots from blue ($\lambda_{\text{max}} \sim 450$ nm) in low concentrations to green ($\lambda_{\text{max}} \sim 525$ nm) in higher concentrations is observed. This effect may be attributed to reabsorption effects of short wavelength light or distinct luminescent species with different self-quenching properties. As such, this behavior could be the basis for several (bio)analytical applications utilizing this effect which is unique to Cdots. Via CLSM imaging, it is shown that normal rat kidney cells incubated with Cdots of different concentrations exhibit varying colors depending on the local Cdot concentration and the laser excitation wavelength.

### Keywords:
- Bioanalytical
- Fluorescence
- Imaging
- Nanotechnology

### Application Code:
- Nanotechnology

### Methodology Code:
- Fluorescence/Luminescence
Nanotechnology became most reliable system for environmental and for biomedical applications within the last decade. This advancement shifted the focus on small carriers to increase the efficiency of the drugs and as a catalyst. Among these, gold nanoparticles (GNPs) found to have profound biomedical and catalytic applications. Our main research focuses on designing gold nanoparticles (GNPs) capped with range of molecules for various biomedical such as enhanced antibacterial, antidiabetic, anticancer activity and catalytic application. We have been successful in synthesizing GNPs using carbohydrates, antibiotics, anti-diabetic and anticancer drugs. The GNPs are characterized by various analytical techniques such as TEM, SEM, FTIR, UV-Vis, TGA, ICP-OES, MALDI-MS and DLS. Antibiotic GNPs are evaluated for their antibacterial activity against Gram-positive and Gram-negative bacterial strains using broth dilution and colorimetric assays. Anti-diabetic GNPs are evaluated using alpha-glucosidase and alpha-amylase assay. Anticancer GNPs are evaluated in various cancer cell line including HeLa cells. Results showed enhanced antibacterial, anti-diabetic and anticancer activity in comparison to the pure drug by itself. We also synthesized carbohydrates capped GNPs for their use as an effective catalyst for industrial applications. We are currently evaluating the effect of size of GNPs on the antibacterial and catalytic properties which will be followed by in-vivo studies and wide array of catalytic testing. Results of these studies not only provide us a novel synthesis method but also yield an efficient drug carriers as well as catalytic agents. This research was support by KY NSF EPSCoR Grant 0814199, WKU research foundation.
Silver nanoparticles (Ag NPs) have posed a wide range of potential applications including serving as therapeutic agents and ultrasensitive optical imaging probes. As Ag NPs have also been used in consumer products, potential release of Ag NPs to aquatic environments could lead to adverse health impacts on human. In this study, we have synthesized and characterized purified and stable silver nanoparticles and then studied their effects on embryonic development using zebrafish embryos as in vivo model organisms. To determine their specific effects on various developmental stages and their related mechanisms, we incubated the embryos with Ag NPs for two hours, and characterized their effects upon embryonic development over time using a wide variety of bioanalytical tools. We also used dark-field plasmonic optical microscopy and spectroscopy to quantitatively characterize single NPs embedded in the tissues of development zebrafish. We found that the effects of NPs on embryonic development highly depended upon embryonic developmental stage, incubation time, the size and dose of NPs.

Keywords: Bioanalytical, Clinical/Toxicology, Imaging, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Sensors
Gold nanorod (GNR) is at the center of attention in biological applications spanning from biosensing to drug delivery. To be successful, conjugation of biomolecules with GNRs is the crucial basis. Current functionalization methods are often problematic, involving multi-step nanoparticle modification to replace surfactant bilayer, delicate nanoparticle protection during surfactant exchange, and material loss due to inevitable aggregation. Instead of intensive surface modification of GNRs, we demonstrated a facile method to functionalize gold nanorod surfaces via covalent Au-S bonds by thiolating receptors. The resulting GNR-bioconjugates showed superior stability and biofunctionality. To demonstrate the practical use, we developed a label-free nano-biochip with optical transduction based on surface plasmon resonance shift of GNRs. The antibody thiolation facilitated a straightforward biochip functionalization with anti-human IgG molecules, which can detect human IgG targets in a chip format with high sensitivity and specificity. Compared to electropolymeric coating to functionalize the GNR, our method exhibited a five-fold enhancement in the spectral sensitivity to refractive index change caused by the target binding. This is due to the significant shortening of the distance between binding events to the optical transducer surface (i.e. GNR sensor) as a result of CTAB replacement by the thiolated antibody moieties. Since gold nanorod is a material of great interest in current research, this work to simplify the biofunctionalization in a simple and efficient fashion is timely and universal for various applications.

Keywords: Bioanalytical, Biosensors, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Other
Nanoparticles (NPs) possess unique physical, surface and chemical properties, offering the possibility of being used as optical and photonic probes for imaging and sensing, as carriers for smart drug delivery, and as medicines to treat a wide variety of diseases. To rationally design the optimum sizes of NPs to achieve highest efficacy of therapeutic effects, we have synthesized and characterized several different sizes of NPs, functionalized them with therapeutic agencies and studied the dependence of efficacy of nano-carriers upon the size of NPs and dose of therapeutic drugs. The updated results and applications will be presented. The work is supported in part by NSF (CBET 0507036) and NIH (R01 GM0764401; 3R01 GM076440-04S1).
Structural biology underpins rational drug design, with crystallography serving as the most widely used method of high-resolution structure determination. The difficulties associated with obtaining large, well-diffracting crystals and X-ray damage have been the biggest limitations of traditional protein crystallography. Serial nanocrystallography overcomes those limitations by focusing on protein nanocrystals and has been shown to be useful in solving the structures of membrane proteins. As a result, the need for fast and selective characterization of protein nanocrystals has arisen.

Second harmonic generation correlation spectroscopy (SHG-CS) is demonstrated as a nondestructive and selective approach for screening different crystallization conditions and for assessing crystal quality prior to exposure to X-ray free-electron laser source. The presence of nanocrystals and the size distribution can be retrieved from the intensity fluctuation of the SHG signal through autocorrelation analysis as particles diffuse through the focus of the laser. Unlike dynamic light scattering, the intrinsic selectivity of the second order nonlinear optical process provides SHG-CS the ability to distinguish well-ordered structures like nanocrystals from conglomerated protein and amorphous aggregates. Custom built instrumentation and software with real time data analysis have been developed to perform SHG-CS measurements. Validation of SHG-CS was performed using BaTiO3 nanoparticles ranging from 200nm to 500nm, and was shown to provide good agreement between the measured correlogram and the theoretical model. SHG-CS was extended to the detection and characterization of protein nanocrystals.

Keywords: Materials Characterization, Nanotechnology, Particle Size and Distribution
Application Code: Nanotechnology
Methodology Code: Other
Silver is used for many uses including purification of drinking water to coatings for medical and industrial applications to take advantage of its antibacterial and antimicrobial abilities. This precious metal while effective at sanitizing is toxic in higher concentrations to organisms in the environment. The use of silver in nanoparticle form is gaining popularity in consumer products and in turn are becoming a threat to the environment with more rigorous usage. These particles enter the waterways through the wash or more directly through the discard of silver coated items. The understanding of silver's interactions with higher plants used for phytoremediation purposes is lacking without further research especially on the cellular response within the plant in the presence of reactive oxygen species. These reactive species are essential to several biological process. However, the overproduction of these reactive oxygen species can cause major oxidative stress leading to cellular damage that may cause cell death or irreversible cellular damage.

This research looks into the production of reactive oxygen species within the leaves of Pistia stratiotes, a plant commonly used for phytoremediation, caused by the presence of silver nanoparticles and silver(I) with various environmental cations using fluorescence detection of oxidative stress.

Keywords: Fluorescence, Nanotechnology, Trace Analysis, Water
Application Code: Nanotechnology
Methodology Code: Fluorescence/Luminescence
Sample preparation for global metabolomics of human plasma is currently predominated by methanol and methanol/ethanol precipitations which produce samples comprised mostly of medium to high abundance metabolites. Solid-phase extractions remain underused in untargeted metabolomics despite their potential to aid in the detection of low abundance metabolome. In this work liquid and solid-phase extraction methods were systematically compared for the extraction of human plasma by liquid chromatography-mass spectrometry (LC-MS). Complex chemical composition of plasma exceeds quantitative and identification capabilities of LC-MS analytical methods. To address this complexity issue, standard mix enriched with hydrophobic (lipids and fatty acids), neutral (sugars, steroids), cationic (amines), anionic (organic acids) and zwitterionic (amino acids) compounds covering range of logP from -6 to 7 was also extracted using all methods for full recovery studies. Samples were extracted in replicates (n=6) with ethanol-methanol, methanol, methyl-tert-butyl ether (MTBE), methanol-MTBE, divinylbenzene–pyrrolidone, octadecylsilane, divinylbenzene-strong-cation-anion exchange and acrylamide-based size exclusion methods. Samples were analysed on mixed-mode (octadecylsilane / weak anion / weak cation) and HILIC (unmodified silica) chromatographic columns coupled to Orbitrap Velos mass spectrometer. Methanol and its blends quantitatively recover high to medium polarity metabolites, while MTBE extracted non-polar compounds. Solid phase extractions demonstrated compound-specific selectivity and size exclusion extracted variable metabolites smaller than 1.5 kDa. Overall, the study allowed to compare selectivity, ionization suppression, repeatability and absolute recovery of extractions from standard mix and human plasma. The novelty of this work is in improved interpretation and applicability of solid-phase extraction methods in untargeted metabolomics of biological fluids.
For more than two decades, CDC researchers have measured tens of thousands of samples for serum cotinine levels as part of the National Health and Nutrition Examination Survey (NHANES). Challenged with more than double the sample load, we developed and validated a rugged, automated sample-to-answer preparation method using Hamilton STARlet and PerkinElmer Staccato Systems robotics technologies. Analysis of serum cotinine is performed by isotope-dilution UPLC/APCI-MS/MS using a Shimadzu Nexera UPLC System coupled to an AB SCIEX Triple Quad™ 6500.

To our knowledge, we are the first to use a fully automated method in the analysis of serum cotinine in smokers and non-smokers. The automated method includes aliquoting via the Hamilton STARlet followed by on-column liquid-liquid extraction and evaporation of extracts on the Staccato System. The Staccato System integrates sample preparation automation via multi-layer platforms, which host multiple peripherals with flexibility to extend modularity and future modification. A powerful Mitsubishi RV-6SDL S15 6-axis robot integrates these peripherals for multi-task operations. The UPLC-MS/MS design integrates additional technologies, such as an autosampler with direct sampling from 96-well plates, and sub-minute separation is achieved. Moreover, software automation is achieved using Indigo Biosystems Ascent to handle the sample-to-answer workflow from sample batch building to data review and final reporting saving hours/days of labor in data analysis. This innovative, automated method has quadrupled sample throughput to 384 samples per day, reduced sample volume by 60% and solvent usage by more than 88%, and cut consumable costs dramatically with no change to our limit of detection. Overall, this sensitive, accurate, and precise method is the basis for future assessment of cotinine in other matrices, such as saliva and urine, as well as the assessment of other major nicotine metabolites, such as trans-3′-hydroxycotinine.

**Keywords:** Biological Samples, High Throughput Chemical Analysis, Laboratory Automation, Liquid Chromatography

**Application Code:** High-Throughput Chemical Analysis

**Methodology Code:** Sampling and Sample Preparation
New regulations for the analysis of trace metals in pharmaceutical ingredients have generated new challenges for the pre-analytical preparation on the wide range of samples that would undergo testing. For USP 232/233, the demand for a clear process is evident. To obtain high quality multi-element ICP-OES/MS data, several techniques have been used without a solution for all samples. Conventional closed-vessel microwave digestion has been recognized as the most effective technique for the digestion of the widest range of sample types in metals analysis. Unfortunately, pressure and temperature constraints using traditional techniques have complicated the picture: multiple steps, incomplete digestions and specific methods for troubling elements. Single reaction chamber (SRC) microwave technology is a new technique with the capability of digesting several different types of samples simultaneously at temperatures up to 300 degree Celsius and pressures to 199 bar. Utilizing this new microwave design with user feedback, an optimized primer on critical factors in preparation choices as well as a single method solution utilizing SRC technology and ICP-MS multi-element analysis will be presented.

Keywords: ICP, ICP-MS, Pharmaceutical, Sample Preparation
Application Code: Pharmaceutical
Methodology Code: Sampling and Sample Preparation
Solid phase microextraction (SPME) is explored as a sample preparation method for profiling volatile metabolites from biological samples (e.g. blood, urine) by GC and GCxGC. Conventional sample preparation methods for biological fluids can be somewhat complex and laborious, whereas sample preparation using SPME is minimized. With the application of SPME, only a fraction of the sample is selectively extracted and introduced to the instrument. However, this may be a small price to pay for the reduced sample preparation requirements. In this contribution, headspace SPME is examined for analysis of human metabolites in whole blood and urine to simplify the sample preparation procedure. Preliminary studies were conducted on whole bovine blood to minimize sample volume and sample preparation for acid- and base-neutral extractions while minimizing issues associated with clotting. Optimized parameters include sample volume, acid, salt, and dilution volume. Extraction times and temperatures were also optimized and fibre chemistry was also explored, as was the use of vacuum-assisted SPME.
Purpose. The experimental objective was to develop a mechanical device that can generate lifelike pharmacokinetic profiles for any chemical compound (realistic animal alternative – RAT).

Methods. An in vitro model matching the biological characteristics of a rat was developed in order to generate pharmacokinetic (PK) profiles corresponding to the one compartment (1C) and two compartment (2C) models with various routes of administration. The majority of the experimental setups generated first order curves, with one setup used to simulate a zero order curve. The device was entirely mechanical, created with pumps and tubing. The change in compound concentration in the liquid was monitored online using spectrophotometry. At the end of each experiment various PK parameters were calculated along with the relative standard deviation (RSD) associated with each of the 3 trials per experimental setup.

Results. The device successfully generated ten types of realistic pharmacokinetic profiles, with all coefficients of correlation (r²) between the desired models and experimental results higher than 0.99 (most r² were found to be 1).

Conclusions. These results suggest that this device is highly accurate and precise at generating realistic PK profiles, and can be used in research and teaching in analytical chemistry and pharmaceutics.

Funding: Albany College of Pharmacy and Health Sciences

Keywords: Bioanalytical, Biological Samples, Pharmaceutical, Sampling

Application Code: Pharmaceutical

Methodology Code: Sampling and Sample Preparation
The neurotransmitter glutamate is the primary excitatory neurotransmitter in mammalian nervous systems. Determinations of glutamate in the nervous system are essential to understanding neurochemical control. Nonetheless, there is wide disagreement with a fundamental measurement; reported concentrations of glutamate found endogenously in the nervous system vary by greater than three orders of magnitude (nanoM to microM). Glutamate levels are studied in this work with low-flow push-pull perfusion applied to mouse hippocampal slices. Perfusate samples are labeled with a fluorophore and separated with capillary electrophoresis to quantitate amino acids including glutamate. Amino acid levels were monitored for 6 hr periods in order to follow any amino acid fluctuations due to tissue activity or bath superfusion of the tissue slice. Experiments determined effects of probe placement and tissue preparation on the observed glutamate. Basal level glutamate is determined from from mutant mice (xCT) with a knocked-out cystine-glutamate transporter and the background strain mice. The transporter has been hypothesized to regulate the extracellular glutamate levels. Results demonstrate the capability of low-flow push-pull perfusion for sampling from relatively thin tissue slices and qualitative analysis of electropherograms shows good similarity to samples collected in vivo. There is a significant difference between the glutamate levels 4.90 (± 1.1) & 1.9 (± 0.5) µM for wild type and xCT (p = 0.0001) mice, respectively. In contrast, no differences are seen in basal levels of glutamine, aspartate and glycine. Glutamate levels over time show that there are significant decreases of basal glutamate in wild type but not xCT slices over the course of 6 hours of sampling. These experiments show a sampling approach that is applied to a tissue slice preparation from which low nM basal glutamate levels are typically reported in contrast to the low microM levels observed here.

Keywords: Amino Acids, Bioanalytical, Capillary Electrophoresis, Small Samples
Application Code: Neurochemistry
Methodology Code: Sampling and Sample Preparation
The direct analysis of tissue tissue samples by matrix-assisted laser desorption ionization imaging can be challenging both due to the requirements for addition of matrix to the tissue as well as limitations due to dynamic range and sample complexity. We are developing a laser ablation to selectively transfer biomolecules from a thin tissue sections using an infrared laser. After the biomolecules have been removed from the tissue and deposited on a separate target, they are analyzed using MALDI imaging. The current work is aimed at the detection of in-situ digested peptides in rat brain tissue sections. The tissue section is mounted on a microscope slide and trypsin digestion is performed after elimination of salts and lipids with serial washing steps. The receiving slide is a nitrocellulose coated indium tin oxide conductive slide. The two slides are mounted on a two-axis translation stage with an 80 µm gap between them. The slides are translated at a speed of 30 µm/s so that the laser irradiates and transfers molecules from a selected area of the tissue. Tryptic peptides are transferred with an IR laser at 3 µm. After transfer, a matrix is sprayed onto the receiving slide and MALDI imaging is performed. With the sample transfer method, we are able to transfer the digested peptides from a tissue section to a target slide with high efficiency. Also, ion suppression due to tissue components is reduced. Ongoing research is being directed at optimizing the spatial resolution of the transfer and selective transfer of tissue biomolecules.

**Keywords:** Biological Samples, Imaging, Laser, Mass Spectrometry

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
We developed a two-dimensional (2-D) photonic crystal lectin sensing material that utilizes light diffraction from a 2-D photonic crystal attached to the surface of a mannose monomer containing hydrogel. Lectin-carbohydrate interactions create hydrogel crosslinks that shrink the hydrogel volume and decrease the 2-D array particle spacing. The lectin sensing hydrogel was prepared through copolymerizing a solution of acrylamide, acrylic acid, N, N’-methylenebisacrylamide, and allyl-modified mannose onto the 2-D array on a glass slide. This mannose containing 2-D photonic crystal sensor detects Concanavalin A (Con A) solution through shifts in the 2-D diffraction wavelength. Con A concentrations can be determined by either measuring the diffracted wavelength, or visually determined from the change in the sensor diffraction color. The concentrations are easily monitored by measuring the 2-D array Debye diffraction ring diameter with a green laser pointer. Our observed detection limit for Con A is 0.02 mg/mL (0.7 M). The 2-D photonic crystal sensor is selective for mannose-specific lectin sensing. The 2-D photonic crystal sensors are completely reversible and can monitor Con A solution concentration changes.

This work was financially supported by HDTRA under grant no. 1-10-1-0044.
Thiamine (vitamin B1) deficiency is a major cause for high mortality in fish of commercial and sport-fishing interest in the Great Lakes and the Baltic Sea. Its deficiency has been associated with poor immune system development and survival rate of fry, as well as reduced visual acuity, neurological symptoms, and muscle weakness in adult fish leading to their inability to migrate during spawning. Its deficiency is also known to cause neurological and cardiovascular problems in other species, including humans. This molecule has recently also been identified as an important nutrient in algal bloom proliferation. Current procedures for thiamine quantification rely on extensive sample preparation and formation of the fluorescent oxidation product, thiochrome, prior to chromatographic analysis. The cost and time required for these procedures limits data available for informed decisions critical to fish health by the fisheries industry and researchers alike. A high-throughput assay for thiamine quantification with specificity provided by biorecognition is thus needed and was the objective of this work. We have previously demonstrated that periplasmic binding proteins can exhibit exceptional specificity and can be used in analytical methods for small-molecule targets not amenable to antibody-based detection. Here, thiamine periplasmic binding protein (TBP) conjugated fluorescent dye-encapsulating liposomes, as an alternative to traditional enzymatic amplification, served to provide recognition and signal amplification in a competitive microtiter-plate assay for thiamine. Results and challenges to development of a total thiamine assay in fish eggs will be presented.

This work was funded by the Great Lakes Fisheries Trust.

Keywords: Bioanalytical, Environmental Analysis, Fluorescence, Immunoassay
Application Code: Environmental
Methodology Code: Sensors
Brevetoxins (BTXs) are very potent marine neurotoxins that increased in geographical distribution in the past decade causing the illness clinically described as neurological shellfish posining (NSP). The ethical problems as well as the technical difficulties associated with the currently employed analysis methods for marine toxins are encouraging the research for suitable alternatives to be applied in a regulatory monitoring regime. Here, we report an electrochemical biosensor platform for BTX-2 detection using aptamer as specific receptor. Using in vitro selection, high affinity DNA aptamers to BTX-2, were successfully selected for the first time from a large pool of random sequences. The binding of BTX-2 to aptamer pools/clones was monitored using fluorescence and electrochemical impedance spectroscopy (EIS). The aptamer BT10 exhibited the highest binding affinity, with a dissociation constant of 42 nM. The effect of the incubation time, pH and metal ions concentrations on the aptamer-toxin binding was studied. The aptamer BT10 was used to construct a label-free competitive impedimetric biosensor for brevetoxin detection showing good sensitivity. A high degree of cross reactivity of the selected aptamer to the two similar congeners, BTX-2 and BTX-3 was observed, whereas no cross reactivity to other marine toxins was obtained. Moreover, the aptasensor was applied for the detection of BTX-2 in spiked shellfish extracts showing a very high recovery percentage. We believe that the continual emergence of novel high-affinity aptamers will open the way to a variety of biosensing architectures, particularly for small-molecule toxin detection in complex samples.

The authors would like to thank NSERC for funding.
Fluorocarbon solvents have low intermolecular forces and are both lipophobic and hydrophobic, causing low solubility for nonfluorous compounds. However, solutions of fluorous carboxylic acid in fluorous solvent have been shown to selectively extract organic bases through hydrogen bonding and ionic exchange. This research focuses on the interaction between perfluorodecanoic acid (PFDA) and pyridine in HFE-7500 (3-ethoxy-1,1,1,2,3,4,4,5,5,6,6,6-dodecafluoro-2-trifluoromethyl-hexane). This is a complicated titration composed of PFDA monomer and dimer, free pyridine, and three different complexes consisting of one, two, and three PFDA to one pyridine, making analysis difficult. Isothermal titration calorimetry was used to measure equilibrium constants and enthalpies.

This system contains eight unknowns: four equilibrium constants and four enthalpies. Reducing the number of unknowns will provide more accurate estimates of equilibrium constants and enthalpies. Thus, we have used MATLAB to determine conditions under which a smaller number of equilibria are relevant. Four sets of conditions, the most complex of which has four unknowns, provide good precision for all eight unknown parameters.

A comparison is being made between the equilibrium constants and enthalpies from ambient and dry experiments performed under a nitrogen atmosphere. Differences have been seen in: dimer enthalpies: -30.98±0.15 kJ/mol (ambient) and -11.89±0.81 kJ/mol (dry) and 1:1 complex enthalpies: -41.94±0.15 kJ/mol (ambient) and -35.81±0.59 kJ/mol (dry). Water content is measured with Karl Fischer titration.

Keywords: Extraction, Materials Science, Sensors, Thermal Analysis
Application Code: Materials Science
Methodology Code: Sensors
Binding interactions at the cell membrane interface are notoriously difficult to study. This talk will describe a novel integration of lipid bilayer nanodiscs, microring resonator sensor arrays, and a non-linear solution gradient generator that facilitates high throughput studies of the blood coagulation cascade. Calcium ions have long been known to regulate activation and binding of blood clotting factors; however, recent studies suggest an important role for magnesium as well. Using the aforementioned approach, we have probed the divalent cation dependence of Factor X binding to a model cell membrane interface. Importantly, the powerful combination of these technologies allowed for the rapid collection of information-rich, multi-dimensional binding profiles as a function of the divalent composition in the buffer. Equilibrium binding affinities were determined over a wide range of conditions showing that magnesium plays a key role in stabilizing Factor X lipid bilayer interactions, consistent with those found physiologically.

Funding provided by NIH National Institute of General Medical Studies

Keywords: Bioanalytical, Biomedical, Biosensors, Biotechnology
Application Code: Biomedical
Methodology Code: Sensors
Designing displacement-dependent nucleic acid biosensors with selectivity to a given analyte is dependent on conformational changes, thermodynamics, and kinetics of the displacement mechanism. We recently developed an innovative biosensor that uses a competitive displacement mechanism composed of a unique self-complementary reporter partially bound to a probe selective for a microRNA (miRNA) analyte. The analyte will displace the reporter from the reporter-probe complex to form a more thermodynamically stable probe-analyte complex and a free reporter. The free reporter, which contains dyes on the 5-prime and 3-prime ends of the sequence, folds into a hairpin structure forcing the dyes close to one another causing a dynamic signal change proportional to analyte concentration. The reporter-probe complexes must be robust to be selective for a given miRNA analyte and used in complex systems such as cells or tissues. Using a MATLAB program developed in-house, we are able to isolate potential reporter sequences that minimize off-target binding. We have designed various different reporters against miR29b-1-5p to evaluate which thermodynamic parameters are most important when developing reporter strands. Gibb’s free energy and melting temperature of the hairpin, reporter-probe, and off-analyte binding are evaluated along with the number of reporter hairpins to identify those most likely to give a maximal signal change. To evaluate which parameters lead to optimal reporter strands, the extent of signal change and kinetics upon reporter-probe and probe-analyte binding were investigated. This research gives insight into what metrics are most important when designing reporter-probe biosensors for miRNAs.

Keywords: Bioanalytical, Biosensors, Fluorescence, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Sensors
Sensors capable to assess very minute (0.2-0.5 units) changes of pH may benefit in molecular level understanding of physiological processes involved in cellular proliferation, apoptosis, ion transport, endocytosis, metabolism, and others. DNA quadruplex-based sensing systems have been established for detection of variety of targets from ions and small molecules to proteins. The sensors possess unique specificity and can be integrated with a variety of signal transduction platforms (e.g. optical, electrochemical, QCM, SPR, acoustic, and/or cantilever-based). We demonstrate on the example of DNA i-motif based pH sensors that both response range and response sensitivity of those systems can be tuned via deliberate manipulation of the quadruplex folding cooperativity. The tools of manipulation include the core quadruplex intrinsic structural features as well as rationally included allosteric elements. We achieved an unprecedented precision of transition point tuning (i.e. down to 0.1 pH units) and response sensitivity overcoming the Henderson-Hasselbalch limit associated with conventional molecular sensors (i.e. width of transition between 10 and 90 % unfolding down to 0.2 pH units and tunable) for the particular systems tailored to respond in the physiologically meaningful pH range. Further, we showed that the sensing approach can be successfully integrated with fluorescent signal readout resulting in a biocompatible detection/imaging platform. The major advantage of the presented i-motif based system over conventional strategies employed for pH sensing is the capability to tune both the response range and/or response sensitivity with the high precision. Those qualities are expected to be instrumental in development of relevant bioanalytical monitoring tools.

Abstract Text

Sensing systems capable to assess very minute (0.2-0.5 units) changes of pH may benefit in molecular level understanding of physiological processes involved in cellular proliferation, apoptosis, ion transport, endocytosis, metabolism, and others. DNA quadruplex-based sensing systems have been established for detection of variety of targets from ions and small molecules to proteins. The sensors possess unique specificity and can be integrated with a variety of signal transduction platforms (e.g. optical, electrochemical, QCM, SPR, acoustic, and/or cantilever-based). We demonstrate on the example of DNA i-motif based pH sensors that both response range and response sensitivity of those systems can be tuned via deliberate manipulation of the quadruplex folding cooperativity. The tools of manipulation include the core quadruplex intrinsic structural features as well as rationally included allosteric elements. We achieved an unprecedented precision of transition point tuning (i.e. down to 0.1 pH units) and response sensitivity overcoming the Henderson-Hasselbalch limit associated with conventional molecular sensors (i.e. width of transition between 10 and 90 % unfolding down to 0.2 pH units and tunable) for the particular systems tailored to respond in the physiologically meaningful pH range. Further, we showed that the sensing approach can be successfully integrated with fluorescent signal readout resulting in a biocompatible detection/imaging platform. The major advantage of the presented i-motif based system over conventional strategies employed for pH sensing is the capability to tune both the response range and/or response sensitivity with the high precision. Those qualities are expected to be instrumental in development of relevant bioanalytical monitoring tools.

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Keywords: Bioanalytical, Biosensors, Fluorescence
Application Code: Bioanalytical
Methodology Code: Sensors
Stir bar sorptive extraction (SBSE) is a solid phase technique for extraction of hydrophobic analytes from aqueous matrices. SBSE has achieved widespread use among biological, agricultural, environmental laboratories and now in environmental human health by improving method runtime, sensitivity, recovery, and precision over existing methods of extraction, such as. Electromagnetic Assisted (EMA) extractions have been used for many decades in the form of microwave digestion and extractions to determine inorganic species, for example. This research was the first to investigate the use of electromagnetic adsorption to significantly reduce the time required for extraction equilibration. Using extraction equilibrium as an endpoint, this research compared sensitivity, extraction temperature, and extraction time of traditional SBSE and EMA-SBSE. Total extraction time was significantly reduced by approximately 90% and increased extraction recovery for the analytes using EMA. The mechanism governing the improved extraction time was investigated using laser-enhanced ionization to determine penetration depth of each analyte into the polymeric extraction phase at varying time intervals. This research has produced a novel extraction method that significantly reduced extraction time and can be paired with standard gas or liquid chromatography. Furthermore, the investigation of the increased extraction efficiency of EMA-SBSE has resulted in a greater understanding of the mechanism through which EMA interact with polymeric solid phase extraction materials.

Keywords: Environmental/Waste/Sludge, Extraction, Laser Desorption, Microwave
Application Code: Process Analytical Chemistry
Methodology Code: Mass Spectrometry
Application of Mass Spectrometry

Quantification of a Biomedically Important Analyte by Enhanced Laser Ionization QTOF-Mass Spectrometry with Speciated Isotope Dilution

Quantitative and effective techniques for blood sampling and analysis are required to meet the growing needs of the medical and bioanalytical communities. Traditional blood draws are inherently used because of the amount of blood typically withdrawn from the patient and most of this blood is discarded. Dried matrix spot (DMS) greatly reduces the volume of collected blood in the sample, thus enabling more frequent and easier procurement with a finger stick instead of a venous blood draw. An additional benefit is the ability to transfer DMS across international borders. Development of the ability to quantitate DMS analytes using mass spectrometry is of importance to bioanalytical research and medicine. Traditional DMS workflows require tedious sample preparation and is more time consuming than direct analysis outlined in this study. Traditional DMS analysis techniques require punching out and extracting the analytes of interest off the card then perform liquid chromatography on the sample prior to mass spectrometric detection. The present study involves the technique of laser-enhanced ionization of the biomedically important peptide, glutathione (GSH). GSH and GSSG (oxidized glutathione) ratios are of particular importance because the ratio is a biomarker for the robustness of the immune and detoxification systems in the human body. This research was performed using an excimer laser enhanced ionization source coupled to a quadrupole time-of-flight (QTOF) mass spectrometer. Laser ionization via matrix-assisted laser desorption/ionization (MALDI) could prove to be a valuable quantitative alternative method for DMS analysis and medical measurements. By coupling MALDI with QTOF mass spectrometry in conjunction with Isotope and Speciated Isotope Dilution Mass Spectrometry quantification can be enabled.

Keywords: Laser, Mass Spectrometry, Quantitative, Speciation
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Electrospray ionization (ESI) has become a staple in the field of mass spectrometry (MS), but its requirement for high voltages makes it less than ideal for field analyses and coupling to miniature MS systems. In recent years, many novel alternative ionization techniques have surfaced, including, but not limited to, desorption electrospray ionization (DESI) (Takáts et al. Science 2004), Venturi easy ambient sonic spray ionization (V-EASI) (Santos et al. Analytical Chemistry 2011), and sonic spray ionization (SSI) (Hirabayashi, A.; Sakairi, M.; Koizumi, H. Anal. Chem. 1994). While these are all excellent ionization techniques, they each require high voltage or compressed gas. Here we present an easy to operate, cost effective ionization technique, spray pump ionization (SPI), that eliminates the need for voltage or gas flow, and demonstrate its utility for analysis of pharmaceutical, peptide, and protein ions.

Mass spectra for pharmaceutical and peptide samples were obtained using a linear trap quadrupole (LTQ)-Orbitrap mass spectrometer. For ESI and SSI, flow rates were set as follows: sample gas at 10 µL/min, sheath gas at 60 AU, and auxiliary gas at 6 AU. Sheath and auxiliary gasses were turned off for SPI experiments and spray voltage was set to 0 kV for both SPI and SSI.

Figure 1a shows the SPI mass spectrum of sildenafil. SPI generated similar spectra to SSI (Fig. 1b) and ESI (Fig.1c) in both positive- (Fig. 1, top) and negative-ion (Fig.1, bottom) modes. While SPI generates lower signal abundance than ESI, our preliminary results suggest that SPI is a softer ionization approach than ESI. Additional examples of the utility of SPI for peptides and proteins will be demonstrated.

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Abstract Text
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Keywords: Bioanalytical, Biological Samples, Sample Preparation, Tandem Mass Spec
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) is an extremely important method for analysis of biomolecules such as proteins and peptides, as well as synthetic polymers. However, MALDI has been very limited in the analysis of small molecules due to the interference of matrix clusters in the low mass regions of MALDI mass spectra. In this work, nanostructured surfaces (nanopillars) are used to effectively absorb laser energy for the vaporization, desorption and ionization of analyte molecules, leading to detection with high sensitivity, resolution and mass accuracy, without any interferences of cluster peaks. The nanopillars have dimensions matching the laser beam wavelength, and can therefore couple laser light very effectively into their local environment. This confinement effect results in electromagnetic field enhancement, high heating rates and prolonged interaction times all of which promote ionization. The excellent performance of these nanopillars is demonstrated using a range of relatively polar small organic molecules, with significant improvement over conventional MALDI in terms of performance and ease of use. These improvements make the use of laser desorption ionization mass spectrometry (LDS-MS) attractive for small molecule high throughput screening (HTS), metabolomics, and mass spectrometry imaging applications. Nanostructures such as these, with well-defined post diameter, periodicity and aspect ratio have been reported in the past, patterned using e-beam lithography, a very expensive and slow process. Here, deep UV projection lithography is employed to achieve the same quality of structures, using a process that is inexpensive and amenable to mass production of the devices.

Keywords: High Throughput Chemical Analysis, Mass Spectrometry, Metabolomics, Metabonomics

Application Code: High-Throughput Chemical Analysis

Methodology Code: Mass Spectrometry
In this study, DART-TOFMS was used to quantitatively analyze the ergosterol content in wild-type and mutant strains of the fungus Fusarium verticillioides growing in and on cracked corn. This analysis, in turn, provided a method for the determination of mycelia mass growing in and on the corn. Samples of cracked corn infected with F. verticillioides were dissolved in a chloroform/methanol mixture with added internal standard. This mixture was used for quantitative DART-TOFMS analysis and the concentration of ergosterol present was determined using an internal-standard calibration curve. The ergosterol content of a sample could be directly correlated to mycelia mass present on the corn, and was quantified using a calibration curve created by measuring ergosterol concentrations in liquid cultures grown under similar conditions. Ergosterol quantification using a DART-100 ionization source was achieved using a custom mount that allowed repeatable analyte sampling in the ion beam. Cholesterol (369.34 Da) was used as the internal standard due to its similar molecular weight (379.33 Da) and structure to ergosterol. Cholesterol was also selected due to its absence in both plants and fungi, whereas ergosterol occurs only in fungal cell membranes. This approach detected ergosterol, and by extension the presence of fungi, on corn. Using appropriate calibration procedures, the mass of fungi growing on or in a plant can be determined. Preliminary data suggest ergosterol concentrations measured in this manner can be employed as a metric for mycelia mass which can be applied to perform mass normalizations for improved quantitative analyses on other solutes in a fungal system, such as sugar alcohols.
Ion mobility (IM) spectrometry is gaining a lot of attention in analytical chemistry due to recent technical advances, which enable to conduct demanding experiments and develop new applications ranging from small molecules to biomacromolecules. Differential mobility spectrometry (DMS) is a variant of IM based on the use of two parallel conductive plates with applied asymmetric radio-frequency (RF) field, which is used in our work to investigate the separation of triacylglycerol (TG) regioisomers.

Traditionally, the separation of TG regioisomers has been achieved by silver-ion chromatography, which requires long gradients (i.e., hours). An alternative MS-based method uses ratios of fragment ions to determine the relative abundances of TG regioisomers in natural mixtures. Here, we present a new fast method for separating and quantifying ratios of TG regioisomers using DMS.

An AB SCIEX QTRAP® 6500 system with SelexION™ was used for these analyses of standard solutions and real biological samples using the direct infusion analysis. Silver ions were added to the solution to form adducts with TG molecules. Multiple reaction monitoring and MS/MS experiments of pure standards and their mixtures were conducted to confirm the identity of DMS-separated compounds.

We have successfully separated four regioisomeric pairs containing one (SOS/SSO, POP/OPP) or two (OSO/SOO, OPO/OOP) double bonds with the addition of silver ions; S means stearoyl (C18:0), P is palmitoyl (C16:0) and O is oleoyl (C18:1\(^9\)). The methodology was applied to a real sample of animal fat to test the applicability of the method for the identification and quantitation of regioisomers in natural samples.

We present a new approach to determine TG regioisomers using DMS. The DMS-based separation yields a marked reduction in the analysis time (i.e., seconds), compared to traditional alternatives. In addition, it offers the possibility of another dimension in the separation of lipids in LC/MS analyses.
Novel Aspect
As a result, an optimized vacuum system with all necessary components: chambers, backing/turbomolecular pumps, gauges and peripheral devices is introduced.

Introduction
A vacuum is required in most analytical devices. The requirements of modern applications are constantly increasing. These requirements concern performance, quality, cost and size. Pfeiffer Vacuum assists in meeting these requirements with customized solutions.

Methods
The poster shows the development of an abstract technological vacuum requirement into an optimized extensive vacuum solution. This exhibits the technological possibilities and versatile approaches, which are developed in close collaboration with the customer. In the first step, a concrete vacuum diagram, using the example of a multi-chamber system, is developed from the requirements. Following this, further general requirements are specified. Using various calculation methods, components from our modular system are optimally selected and combined. This shows how different methods, such as the Monte Carlo method, the CFD method, the finite element method and analytical calculations are applied.

Preliminary Data
Not only the vacuum technology but also mechanical properties, service life and reliability are considered. An optimized vacuum system is produced from these results. This shows how the other components of the overall system are structured and synchronized.

From the overall analysis, important parameters, such as power input, cooling requirements, weight and size can be determined and adapted to the customer requirements.

Keywords: Instrumentation, Mass Spectrometry
Application Code: Other
Methodology Code: Mass Spectrometry
Nowadays, consumers keep away from taking foods that contain high salt (sodium chloride), due to adverse health effects of current dietary sodium intake. Thus, this study was carried out to investigate the influence of salt reduction on quality of brined white cheese.

The effect of varying salt concentrations of 1, 3, 5 and 7.5% on the quality of white brined cheese was examined by SPME-GC–MS, RP-HPLC, and urea-polyacrylamide gel electrophoresis (urea-PAGE). The cheese samples were stored at 4-6 °C and chromatographic analysis were performed at 7., 30., 60., and 90. days of ripening during the storage period. Proteolysis in the cheeses was monitored by HPLC and urea-PAGE analysis and the flavour compounds of the cheeses were determined by SPME-GC-MS. A method optimization process of the SPME procedure for white cheese was carried out by considering different fiber types, extraction temperatures, and sample mass.

The cheeses ripened in varying salt concentrations did not differ in hydrolysis of $\alpha$- and $\beta$-caseins. When the chromatograms obtained from HPLC were examined by principle component analyses (PCA), it was observed no significant difference in peptide profiles of the cheeses ripened in different salt concentrations. A specifically aimed SPME-GC-MS method was assessed in order to determine the volatile compounds of white brined cheeses. Consequently, CAR/PMDS fiber, extraction at 45 °C, and 10 g of sample were selected as the optimal experimental conditions. Twenty two volatile compounds were detected in the cheeses, consisting of 5 alcohols, 9 carboxylic acids, 2 esters, 5 ketones, and a terpene by SPME-GC-MS. This study has shown that the texture and flavour of the low salted cheeses has been conserved during ripening period because the low salt concentration of brine has not affected proteolysis in the cheeses. Therefore, the salt concentration can be reduced in white cheese production because of its contributions to health.

**Keywords:** Chromatography, Food Science, GC-MS, HPLC

**Application Code:** Food Science

**Methodology Code:** Mass Spectrometry
The extravasation of the administrated nano-drug carriers played a crucial role in the outcomes of their distribution in the target and non-target organs as well as pharmaceutical efficacy and side effects. To evaluate the extravasation behaviors of the gold nanoparticles (AuNPs), currently the most popular drug delivery system, in a mouse tumor model, in this study we introduced the push-pull perfusion (PPP) as a means of continuously sampling the tumor extracellular AuNPs. To facilitate the quantification of the extravasated AuNPs by inductively coupled plasma mass spectrometry, a novel online open-tubular fractionation scheme was also developed to allow the interference-free determination of the sampled extracellular AuNPs from the coexisting biological matrix. After optimizing the flow-through volume and flow rate of this proposed fractionation scheme, (i) the system’s temporal resolution was 7.5 h⁻¹, (ii) the stability presented by the coefficient of variation was below 10% (6-h continuous measurement), and (iii) the detection limits were in the range of 0.057-0.068 [µg L⁻¹] for the administrated AuNPs. Following by an intravenous dosage of AuNPs (0.3 mg kg⁻¹ body weight), the in vivo acquired profiles indicated that the pegylated AuNPs showed more tendency toward extravasating into tumor extracellular space. A higher accumulation of nanoparticles in the whole tumor tissues was also observed for pegylated AuNPs than that of non-pegylated ones. Overall, the experimental results confirmed that the pegylation would potentially promote the extravasation and accumulation of AuNPs for nano-drug delivery applications.

Keywords: Analysis, Automation, On-line, Particle Size and Distribution
Application Code: Pharmaceutical
Methodology Code: Mass Spectrometry
Application of Mass Spectrometry

Mapping O-GlcNAc Modification Sites in ABL2 by Tandem Mass Spectrometry

More than half of the human proteome is regulated by the post-translational modification, O-linked N acetylglucosamine (O-GlcNAc), which is related to a number of human diseases such as diabetes, Alzheimer’s disease, and cancer. The goal of this study is to develop a proteomic mass spectrometry (MS) approach to study sites of O-GlcNAc modifications on proteins produced in E. coli. MS experiments are performed on a nano-electrospray ion trap mass spectrometer with electron transfer dissociation (ETD) and collision induced dissociation (CID) to map the modification sites to specific serines and threonines. The system is also equipped with a nano-LC system. Preliminary work has been performed using chymotrypsin digestion using CID fragmentation tandem mass spectrometry. This work is being extended to ETD fragmentation. The O-GlcNAc modified Abelson tyrosine kinase 2 (ABL2) protein was produced by co-expression of O-GlcNAc transferase in E. coli. The ABL 2 protein was purified by Ni-affinity chromatography and gel filtration chromatography. Seven (S50, S51, T54, S60, S74, S94 and S99) out of twenty possible residues have been identified with the O-GlcNAc modification. Matrix-assisted laser desorption ionization (MALDI) tandem time of flight mass spectrometer has been used to maximize the sequence coverage up to 84%. The long term goal of this project is to use the E. coli system to generate O-GlcNAc-modified proteins for site mapping and structural analysis by mass spectrometry and nuclear magnetic resonance spectroscopy.

Abstract Text

Identification, Liquid Chromatography, Proteomics, Tandem Mass Spec

Bioanalytical

Mass Spectrometry
This paper explores the use of thermally assisted derivatization techniques. In some cases, derivatization can simplify identification of polymers by providing larger, more descriptive fragments of the polymer when compared to conventional pyrolysis GC/MS. It can also improve chromatography on commonly used low polarity columns by transforming nonpolar molecules to polar ones. Techniques such as thermally assisted hydrolysis and methylation (THM) using tetramethyl ammonium hydroxide, and silylation using HDMS will be demonstrated. THM converts alcohols, amines and acids into methyl esters. Silylation with Hexamethyldisilazane (HDMS) performs the same function, only instead adding trimethyl silyl group to non-carbon atoms containing active hydrogens, and some carbonyl groups. Silylation reduces polarity, enhances volatility, and increases thermal stability, enabling the GC-MS analysis of compounds otherwise not volatile or too unstable. They often allow for better GC separation and the produce more diagnostic fragments in the MS. We will explore derivatization products of natural materials such as oils and waxes, lignin and polysaccharides (cellulose, sugar), and synthetic materials like polyesters, epoxies, polycarbonates and polyurethanes. Derivatization chromatograms will be compared with thermal desorption and pyrolysis chromatograms in which no derivatization was used.
Application of Mass Spectrometry

An Alternative to DIP/DEP: Pyroprobe Coupled to High Resolution Time-of-Flight Mass Spectrometry

Direct probe analysis is a common option for rapid characterization of non-GC amenable liquids or solids having low vapor pressures. These materials include, but are not limited to, organometallics, drug substances, pharmaceutical powders, polymers, and natural substances. The ability to perform these analyses is essential for many core mass spectrometry labs. The objective of this study was to perform direct probe like analyses of substances utilizing high resolution time-of-flight mass spectrometry in electron ionization (EI) and chemical ionization (CI) modes for compound identification. A CDS 5200 series pyroprobe was coupled to Leco’s Pegasus high resolution time of flight mass spectrometer (HRT) with an uncoated capillary connecting the inlet to the ion source. The benefit of using a pyroprobe to perform this analysis was the added versatility compared to direct probes. Liquids or solids could be introduced via a quartz tube or placed directly onto a ribbon filament and thermally desorbed or pyrolysed. Also, because the pyroprobe is interfaced directly with the GC inlet, it is possible to perform distillation cut type experiments or chromatographic separations if required. The effectiveness of this technique for analyzing sample types commonly submitted to core labs will be presented.

Keywords: Characterization, Mass Spectrometry, Sample Introduction

Application Code: General Interest

Methodology Code: Mass Spectrometry
Many laboratories are adopting GC triple quadrupole MS to access the widely accepted advantages brought by high selectivity detection of target analytes. However, while the advantages of MS/MS scan modes, particularly SRM, is well documented, some regulated methods such as EPA 8270 do not permit its use currently. Laboratories that are accredited for these “prescriptive” regulated methods are then compelled to continue with current technology such as GC single quadrupole MS. This prevents users become familiar with the latest technology and means triple quad technology is unavailable for unregulated sample analysis or special projects. This work describes how GC triple quadrupoles can be used in single quadrupole modes to satisfy current regulations as well as being able to very easily switch to powerful SRM methods when needed, especially in preparation of regulations being updated in future. Also included is some potential applications of mixed MS modes such as simulatenous FS/SRM.

Keywords: Gas Chromatography/Mass Spectrometry, GC-MS, Tandem Mass Spec
Application Code: General Interest
Methodology Code: Gas Chromatography/Mass Spectrometry
The islets of Langerhans are the endocrine portion of the pancreas that help control blood sugar and metabolism through secreted hormones. Several diseases are associated with dysfunctional and unregulated secretion, most notably diabetes mellitus. Here we performed a proteomics analysis of the secretion of cultured intact murine islets under different conditions. These responses help identify how islets respond to differing levels of glucose and lipids.

**Abstract Text**

The islets of Langerhans are the endocrine portion of the pancreas that help control blood sugar and metabolism through secreted hormones. Several diseases are associated with dysfunctional and unregulated secretion, most notably diabetes mellitus. Here we performed a proteomics analysis of the secretion of cultured intact murine islets under different conditions. These responses help identify how islets respond to differing levels of glucose and lipids.

**Keywords:** Bioanalytical, Biological Samples, Mass Spectrometry, Proteomics

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
Insufficient ion mobility (IM) resolving power of Ion Mobility-Mass Spectrometry (IM-MS) systems may hinder separation of isomeric species with similar collision cross sections (CCSs) and result in erroneous analyte characterization. Recently, we showed that unresolved IM profiles and collision-induced dissociation (CID) mass spectra could be deconvoluted using chemometric analysis of post-IM/CID MS data [1]. Here, we report collision-energy resolved IM deconvolution of a four component peptide isomer mixture with overlapping IM profiles.

IM-MS data were collected using a traveling wave IM time-of-flight (TOF) mass spectrometer operated in positive-ion mode electrospray ionization (ESI). A three or four component mixtures of peptide isomers will be isolated in the quadruple mass filter (positioned prior to the IM cell). Subsequently, m/z-isolated species will be subjected to post-IM/CID as a function of collision energy (i.e., potential difference between the exit of IM cell and entrance of a stacked-ring ion guide assembly positioned between the IM cell and TOF). Our preliminary data show that collision energy corresponding to a 32 V potential difference provided appropriate post-IM/CID MS data for the successful deconvolution of the tertiary mixture. Successful deconvolution represents an energetic “sweet spot” for optimized chemometric analysis of the isomeric species. In this presentation, results from energy resolved deconvolution of various sample mixtures will be discussed.


Keywords: Chemometrics, Data Mining, Mass Spectrometry, Separation Sciences

Application Code: Genomics, Proteomics and Other 'Omics

Methodology Code: Mass Spectrometry
Carbohydrates are characterized by their complex structures and ubiquitous nature. They are involved in a wide range of crucial physiological and pathological cellular events. Screening mixtures of naturally occurring oligosaccharides for specific protein interactions using electrospray ionization mass spectrometry assay will be presented. The mass spectrometry assay boosts many virtues mainly its low sample consumption, speed, simplicity as minimum amount of sample preparation is required with no labeling or immobilization. In this study, the implementation of tandem mass spectrometry in identifying and quantifying oligosaccharides from natural sources as milk will be described. The assay allows the quantification of binding affinities for protein-carbohydrate interaction depending on the abundance ratio of bound to unbound protein directly extracted from mass spectra. The proposed method is extended to study human milk oligosaccharide isomers as lacto-N-tetraose, β-D-Gal-(1→3)-β-D-GlcNAc-(1→3)-β-D-Gal-(1→4)-β-D-Glc; lacto-N-neo-tetraose, β-D-Gal-(1→4)-β-D-GlcNAc-(1→3)-β-D-Gal-(1→2)-β-D-Glc also 2-fucosyllactose, β-L-Fuc-(1→2)-β-D-Gal-(1→4)-β-D-Glc and 3-fucosyllactose, β-D-Gal-(1→4)-(β-L-Fuc-(1→3)]-β-D-Glc. Using collision-induced dissociation (CID), the relative abundance of the isomeric ligands are determined from the abundances of fragment ions produced by CID. Furthermore, ion mobility separation is applied to measure the abundance of the isomers, where Gaussian functions were used to represent the arrival time distributions of each isomer. The reliability of the results extracted from the method is demonstrated using model systems. Different applications beside the mathematical framework for the presented method will be given.

Keywords: Carbohydrates, Mass Spectrometry, Natural Products, Protein

Application Code: Other
Methodology Code: Mass Spectrometry
Ambient mass spectrometry (AMS) is a rapid analytical tool capable of providing comprehensive chemical analyses of reaction mixtures resolved on thin layer chromatography (TLC) plates. User-guided investigation of mass spectrometry imaging (MSI) data for mixture components with known m/z values is generally straightforward; however, spot detection for unknowns is rather tedious and has limited the applicability of mass spectrometric imaging for TLC plate analysis. For complex reaction mixtures, an understanding of all chemical products is necessary for the comprehensive investigation of reaction mechanisms. Therefore, we have developed DetectTLC, an automated approach that identifies m/z values exhibiting spot-like shapes in mass spectrometric images. Furthermore, advances in mass spectrometry instrumentation now allow for multi-modal analysis in which alternating scans are acquired in different modes of operation, i.e. alternating between a low-energy (parent ion) and high-energy (fragment ions) scans. Without precursor mass selection, correlating parent and fragment ions is impossible. DetectTLC is also capable of correlating spots observed in the two modes of operation without precursor mass selection based on the spatial distribution of m/z values. Employing desorption electrospray ionization mass spectrometry (DESI-MS) as the ambient sampling method in conjunction with DetectTLC, we were able to identify and characterize previously unknown products of an abiotic pyrazine nucleic acid synthesis products. This nucleic acid synthesis is of great interest to prebiotic chemists and the origin of life community but the DetectTLC software package is also expected to be extremely useful for any application that benefits from fast TLC separations or for reaction monitoring purposes.

Keywords: Bioinformatics, Imaging, Tandem Mass Spec, Thin Layer Chromatography
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Characterization of the metabolome, the complete suite of metabolites expressed by embryonic cells, is key to understanding healthy embryo development. However, this requires new analytical technologies, particularly those that are highly sensitive, selective to diverse types of biomolecules, and compatible with extremely small sample volumes afforded by single cells. We report here a new approach based on mass spectrometry (MS) to enable profiling metabolites in single embryonic cells of the South African clawed frog (Xenopus laevis), the favorite model in cell and developmental biology. We use this information to uncover, for the first time, cell-type specific metabolic differences in the early developmental stage of the embryo.

Our technology combines microsampling and volume-limited capillary electrophoresis (CE) high-resolution tandem MS that we recently developed. CE-MS achieved a low limit of detection below 10 nM (60 amol) for acetylcholine among other compounds, sufficiently low to assess endogeneous metabolite levels. Embryonic cells were identified and microdissected from 16-cell Xenopus embryos, their metabolomes were extracted, and 10 nL of the extracts were measured by CE-MS. Profiling revealed more than 80 different small molecules that were consistently expressed among the cells, 45 of which were confidently identified as metabolites. Multivariate and statistical analysis of the data revealed differential clustering between cells in the ventral, dorsal, and vegetal regions of the embryo. The finding that embryonic cells have characteristic metabolomes during early development is surprising, and demonstrates that single-cell MS raises a new opportunity to investigate biochemical processes that underlie embryonic development.

Keywords: Capillary Electrophoresis, Mass Spectrometry, Metabolomics, Metabonomics
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Radio-frequency ionization (RFI) is a novel ionization method for mass spectrometry (MS) analysis of volatile organic compounds (VOCs) in positive- and negative-ion modes. We recently reported on RFI/MS and chemometric analysis of VOC mixtures. Despite the unprecedented sensitivity of RFI, mechanism of ion generation in RFI is not well understood. In this presentation, we will provide the first experimental evidence for generation of electrons in RFI. Moreover, we will demonstrate the utility electron capture ionization in RFI.

RFI source consists of a home-built RF power supply for generation of RF signals (~12 MHz, 400 Vp-p) and a set of dipole RFI electrodes. The dipole electrode are placed within the vacuum chamber of a 9.4 T Fourier transform-ion cyclotron resonance (FT-ICR) mass spectrometer and in proximity of an ICR cell. Headspace hexafluorobenzene (C6F6) will be introduced into FT-ICR vacuum chamber from a heated expansion reservoir (150 ºC), transfer line (120 ºC), and pulsed valve (120 ºC).

Following a 2-s electron relaxation time after RF signal application and trapping RFI-generated electrons, C6F6 neutrals were introduced into the vacuum chamber. Resulting negative-ion mode FT-ICR mass spectra showed the presence of C6F6⁻ species. In recent studies and to further confirm that C6F6⁻ species were products of reaction between RF-generated electrons and C6F6, an electron-quenching event was introduced for varied time durations (0-13 ms). Results from negative-ion mode post-RF FT-ICR data that confirm the generation of electrons in RFI will be discussed.

Authors would like to acknowledge the financial support from Baylor University.

References

Keywords: Biofuels, Chemometrics, Mass Spectrometry
Application Code: Environmental
Methodology Code: Mass Spectrometry
**Application of Mass Spectrometry**

**Analysis of Organic Ligands Attached to Gold and Silver Nanomaterials Using Ambient Ionization Mass Spectrometry**

Nanomaterials are being used extensively for industrial and medical uses. Gold or silver nanoparticles can be modified with organic monolayers often containing functional groups for covalent attachment to biomolecules or other organic molecules. The goal of our study was to identify various types of ligands attached to nanoparticles using ambient ionization mass spectrometry. A PerkinElmer DSA Time-of-flight mass spectrometer (TOF) was used for the analysis. The DSA source uses a “field free” corona discharge electron Reagent Ion Generator that ionizes analytes in ambient atmospheric conditions. The sample was layered on a steel mesh and then exposed to the source. The organic capped ligands are released from the nanoparticles due to thermolability of the covalent bonds however, the temperature of the source is not sufficient to volatilize the gold and silver particles themselves. Using accurate mass and isotope profile information provided by the TOF, along with powerful visualization software tools, we were able to confirm the presence of the different types of ligands attached to nanoparticles. Besides using the DSA-TOF as a screening tool for the organic ligands, we were also able to obtain semiquantitative information on the amount of ligand attached to the nanoparticles.

**Keywords:** Characterization, Mass Spectrometry, Method Development, Nanotechnology

**Application Code:** Nanotechnology

**Methodology Code:** Mass Spectrometry
This paper describes the theory of Single Particle-ICP-MS in analyzing Metal Based Nanoparticles using the NexION 350 Syngistix Nano Application Module. Single Particle (SP-ICP-MS) allow the differentiation/quantification between ionic and particulate signals, measures particles concentration (part/mL), ionic concentration (µg/mL), particle sizes, size distribution and explores agglomeration and dissolution kinetics.

SP-ICP-MS is a key analytical instrument in assessing the fate, behavior and distribution of engineered Nanoparticles (ENPs) in several types of matrices (environment, food, etc.), evaluating ENPs bioavailability and bioaccumulation in the biota, and improving bio-labeling capabilities and advancements in the medical field.

Keywords: Characterization, Environmental Analysis, Nanotechnology, Particle Size and Distribution
Metabolomics, the study of small molecules involved in cellular processes, offers the potential for investigating disease pathogenesis and has proven useful in the development of therapeutic targets. Metabolites are a diverse group of compounds with a variety of functionalities including hydroxyl, amine, carboxyl, phosphoryl, and thiol groups. Electrospray ionization mass spectrometry (ESI-MS) is widely used to detect metabolites due to its high sensitivity and ability to generate structural information. However, the structural diversity of metabolites results in unequal ionization efficiency with ESI and consequently varying degrees of sensitivity. These limitations may prevent the detection of molecules present in low abundance in biological samples. Tagging approaches have been used to improve electrospray by allowing charge to be concentrated on the outside of the droplets and enabling more efficient evaporation of mobile phase. Furthermore, highly ionizable tags ensure equal ionization efficiency for all tagged analytes. The goal of this project is to use two cationic hydrophobic tags to label metabolites with hydroxyl, amine, carboxyl, phosphoryl, and thiol groups and improve sensitivity in ESI-MS. This work will make the simultaneous detection of a diverse group of metabolites possible, leading to a more complete picture of the metabolic system of interest.
As the field of nanotechnology grows it becomes crucial to develop and validate methodologies to not only detect but to also characterize these particles. This remains a challenge due to their relatively small size and the complex matrices they may be found in. Single particle inductively coupled plasma mass spectrometry combines the traditional method of metal detection with the new approach of shorter dwell times to detect these nanoparticles (NPs). This technique scans and collects data at much shorter intervals, which allows for the detection of minimal variation in the number of ions striking the detector within a dwell time of milliseconds. This allows the short burst of increased signal contributed by a particle to be distinguished from the constant signal of ions. The intensity of this signal can be used to calculate the size of the contributing particle.

Here, single particle ICP-MS was shown to be a valid and robustness method for the detection, characterization and quantification of PVP-Ag NPs used in a study designed to investigate the effects of cell cultures exposed to this particle. NPs were suspended in solutions of a combination of media, serum and cells to for up to 48hrs to determine if dissociated occurred as well as total ion concentration.

Keywords: ICP-MS, Method Development, Nanotechnology, Trace Analysis
Application Code: Nanotechnology
Methodology Code: Mass Spectrometry
State-of-the-art Proton-Transfer-Reaction Time-of-Flight Mass Spectrometry (PTR-TOFMS) instruments perform with sensitivities of typically 100 - 200 cps/ppbv. Here we present a novel PTR-TOFMS setup that utilizes a Quadrupole ion guide (Qi) instead of a common lens system for a much more effective transport of ions into the mass spectrometer. With measurement data obtained by introducing certified gas standards into the PTR-QiTOF we demonstrate that its sensitivity is up to 4,700 cps/ppbv, i.e. about 25 times more than the best instruments so far. Such outstandingly high sensitivities are of particular importance in fields of application where time per analysis is limited, e.g. flux measurements in atmospheric chemistry and mouth- or nosespace analysis in food and flavor research. Notably, in these fields the chemical environment is usually very complex so that even the selectivity of a high-resolution mass spectrometer is not sufficient. Monoterpenes, for example, are important compounds in atmospheric chemistry as well as in food and flavor research and appear at m/z 137 (protonated monoterpenes). Therefore, we coupled the novel PTR-QiTOF with a FastGC inlet system and analyzed manuka tea and spruce resin, respectively. In the figure below one can see that indeed in normal direct-injection mode all monoterpenes appear indistinguishably at m/z 137. However, after switching the inlet system to FastGC mode the monoterpenes get separated according to their retention times and are easily distinguishable. The FastGC run takes less than 60 s and can even be considerably reduced to quasi-real-time by applying a sophisticated GC pulsing method (patent pending).

Keywords: Environmental Analysis, Food Science, Time of Flight MS, Volatile Organic Compounds
Application Code: Environmental
Methodology Code: Mass Spectrometry
USEPA Method 8260 involves purging analytes out of a water matrix. During a split injection, the sample volatilizes in the inlet and is swept by the carrier gas through the liner onto the GC column with a portion of the sample being split off and sent out the split vent line. The amount of sample reaching the detector is dependent upon the split flow rate. Thus, the higher the split rate, the smaller amount of sample on the column. Furthermore, a higher split ratio minimizes column exposure to moisture. During purge and trap sampling moisture control is crucial to decreasing split flow and enhancing detection limits. This application will explore the effect split ratios have upon USEPA Method 8260 analytes.
We have developed a novel mass spectrometric visualization method to generate pseudo cross-sectional skin images for fast and non-invasive transdermal drug delivery evaluations. Two-dimensional desorption electrospray ionization / mass spectrometry (2D-DESI MS) was utilized to obtain planer distribution information of drug penetrated into stratum corneum (SC).

We have visualized several drugs in SC successfully, but nonpolar compounds such as cholesterol and retinol were not ionized efficiently under typical DESI conditions because of their low proton affinity. To solve this problem, we have focused on reactive-DESI in which derivatization reagent is added to DESI spray solvent to increase their proton affinity.

In this study, we investigated potentials of reactive-DESI for mass spectrometry imaging (MSI) to expand the utility of DESI-MSI for compounds not efficiently. Retinol was chosen as a model compound, which was reacted in the gas phase with betaine aldehyde, a reagent of Reactive-DESI for –OH group(s). Potentials and limitations of reactive-DESI MSI will be discussed in the presentation.

Keywords: Cosmetic, Electrospray, Imaging, Mass Spectrometry
Application Code: Consumer Products
Methodology Code: Mass Spectrometry
Elemental analysis by inductively coupled plasma optical emission spectrometry (ICP OES) can be influenced by variations in operating conditions and matrix effects. These interferences can be efficiently minimized by using the internal standard (IS). Bismuth was recently proposed as a general internal standard for Pb in atomic absorption spectrometric determinations [1]. Considering the possibility of expanding this proposal to other spectroscopy techniques, this work evaluated Bi as internal standard for Pb determinations by ICP OES. A Varian Vista AX ICP OES was used for simultaneous measurements of Bi and Pb at 223.061 nm (I) and 220.353 nm (II), respectively. The influence of variations of the nebulizer gas flow-rate (0.7 - 1.1 L min⁻¹), radio-frequency power (0.90 - 1.25 kW) and sample matrix (34 different samples) on the emission signals for Pb and Bi was evaluated, and similar variations were observed for both elements. Three certified reference materials were analyzed and results found for Pb using IS were in agreement with certified values at the 95% confidence level. The use of Bi improved the RSD from 0.8 - 9.0% (without IS) to 0.2 - 2.9% (IS). Recoveries of spiked samples improved from 56 - 91% (without IS) to 95 - 110% (with IS). These findings indicated the usefulness of Bi as internal standard for broad use in ICP OES for Pb determinations.

The interaction of toxic metals with essential elements could lead to disruption in the homeostasis of essential metals. The study investigated the effect of low level exposure of inorganic mercury to essential elements (Zn, Fe, Se, Mg and Ca) of male and female rats. The rats were exposed to 0.5, 1.0 and 1.5 mg/kg of Hg concentrations for 12 weeks after which blood, liver, kidney, brain, spleen, heart and lungs were removed from the animals and these elements were determined by inductively coupled plasma-mass spectrometer (ICP-MS). The result showed that mercury accumulated in all the organs but more mercury uptakes were observed in the kidney than every other tissues, markedly in females (77.89±7.7 and 23.78±1.59 µgHg/g). The exposure resulted in decrease in Mg, Ca, Fe, and Zn in nearly all the tissues except for brain and liver where Fe increased significantly in both sexes compared to controls(p<0.05). Se behaved differently in kidney and blood, in terms of its mutual competition with Hg. The increase in Hg dose resulted in gradual and significant increase in the concentration of Se in kidney and blood. There was also significant increase in Ca and Mg in lungs of both sexes. No variation was observed with zinc in brain and liver. Most notably, Hg correlated negatively with Ca and Mg. These findings indicate interaction between essential and toxic metals.
Analytical chemistry is an important tool in the mining process. Quantitation of gold, silver or platinum group metals in ore grade material and mapping geochemical composition of rocks are essential to ensure the commercial viability and the environmental impact of mining processes. However, the analysis of geological samples can be very challenging because of their complex, heterogeneous mixtures of minerals. They are often formed from a multitude of small grains, each with a different composition.

Analytical technics such as ICP-MS, ICP-OES and AA provide viable solutions for inorganic analysis. However, they must be simple to use, offering flexibility, reliability, high productivity and accurate results with these challenging sample matrices.

This poster presents analytical results of mining samples by AA, ICP-OES and ICP-MS, focusing on simple sample preparation, method development. It also addresses the issues of accuracy, interference removal and stability. Results of real samples and Standard Reference Materials are shown with discussion.

**Keywords:** Atomic Absorption, Geochemistry, ICP, ICP-MS

**Application Code:** Other

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
Mining activities involve mineral prospecting and exploration which can be quite expensive. A number of mineral prospecting methods which are commonly used include: excavation of soil for analysis, phyto-prospecting and hydrogeochemical prospecting. Ants and termites have been reported to burrow up to 55 metres to water tables. In the process, they bring up debris, which may contain traces of minerals in mineralized areas. Qualitative and quantitative analyses of anthills' and termite mound soil samples can be used as preliminary tests for prospects of finding a given mineral in a particular area. Termitaria sampling has been used in other parts of the Worl such as Australia, Ghana and India. This study sought to determine whether there are significant levels of iron and titanium in anthill soil samples as compared to top soil samples from Kwale, Kathwani and Kithiori, which are mineralised areas in Kenya. Flame atomic absorption spectroscopy was used to determine iron and titanium after carrying out acid digestion of soil samples. It was found in this study that the control samples had lower concentrations of iron for all the three sampling areas; 20.63+_0.29mg/g compared to mean concentration of Fe in anthill samples of 25.30+_0.06 m9/g in Kwale. 90.53+_0.01mg/g compared to 98.53+_0.29 mg/g at kathwana and 82.63+_0.22 compared to 89.48+_0.01 mg/g at Kithiori. Titanium content in termitaria samples was higher than the control samples for all the three areas; 14.9+_0.24 mg/g compared to 14.72+_0.00mg/g at Kwale, Kithiori 18.47+_0.03 mg/g compared to 15.93+_0.24 mg/g at Kithiori and 14.52+_0.03 mg/g compared to 13.13+_0.00 mg/g at Kathwana. These results demonstrate the potential for use of termitaria soil sampling in mineral prospecting. For conclusive results of iron and titanium mineralization, multimedia sampling is recommended.
Investigation into the Presence of Mercury in Game Controllers

The use of game controllers is prevalent with millions of units found worldwide, yet very little research has been conducted into the potential presence of hazardous materials. This study aims to assess the presence of hazardous materials and the potential environmental impact. A preliminary screening involved the dismantling of seven controllers from leading companies within the gaming industry into their individual components before using X-Ray Fluorescence (XRF) which detected mercury in one particular model. Five further controllers of the same model were purchased and the logos analysed using Scanning Electron Microscope (SEM) in order to provide a secondary qualitative measurement. Finally samples were analysed using Continuous Flow Vapour Generation Atomic Fluorescence Spectroscopy (CV-AFS). Initial results from XRF demonstrated the presence of mercury in the logo of a specific controller with further analysis of the sample using SEM confirming the initially findings. CV-AFS was conducted, which produced significant results. It demonstrated that mercury was present throughout the logo at concentrations at the parts per million levels. The high level of mercury discovered in the game controllers could pose a significant source of contamination which could have a potentially damaging impact on the environment through the disposal and degradation of materials in the controllers.

This research will discuss and highlight game controllers as a novel source of major contaminants, such as mercury, within the environment. The use of continuous flow vapour generation atomic fluorescence spectroscopy to measure mercury in complex samples will also be discussed and presented.

Keywords: Atomic Spectroscopy, Consumer Products, Mercury, Microspectroscopy
Application Code: General Interest
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Sample introduction efficiency is very important for analytical atomic spectrometry, especially considering the method sensitivity. In comparison with the conventional nebulization technique, ultrasonic nebulization techniques greatly enhance the sample introduction efficiency from 5 to almost 100% resulting in almost two orders of magnitude improvement in sensitivity [1]. Recently, we have been working on ultrasonic micro-nebulization technique as a sample introduction tool for analytical microwave induced plasma optical emission spectrometry (MIP-OES) when performing of hydride forming (As, Bi, Ge, Sb, Se, Sn), cold vapor (Hg, Cd), transition (Au, Ag, Cu, Mn, Ni, Pb, Zn), noble (Pd, Pt, Rh) and non-hydride forming (Ba, Ca, Li, Mg, Sr) elements determination and speciation analysis. The ultrasonic nebulizer supplied a microliter sample to a quartz oscillator; the hydrides, metal volatile species and aerosols were rapidly transported via stream of Ar carrier to a MIP for simultaneous multi-element determination by OES. A univariate approach and simplex optimization procedure was used to achieve optimized conditions and derive analytical figures of merit (LODs, RSDs). The methodology was validated through determination of elements in several Certified Reference Materials and by the aqueous standard calibration technique. The proposed method was applied to the determination of elements in real samples.

References

Acknowledgements
We thank the financial support from the National Science Centre (NCN), Poland (Grant No. UMO-2012/06/A/ST4/00382).

Keywords: Elemental Analysis, Environmental/Biological Samples, Plasma Emission (ICP/MIP/DCP/etc.), Sample Application Code: Environmental Methodology Code: Atomic Spectroscopy/Elemental Analysis
For the first time, a spatial-heterodyne spectrometer (SHS) is used for standoff laser-induced breakdown spectroscopy (LIBS) measurements. The SHS is a diffraction grating based interferometer with no moving parts. The SHS design offers advantages over dispersive spectrometers including 10-100 times larger acceptance angle and subsequently a much larger field of view, 100-10,000 times higher light throughput, high spectral resolution, and a relatively wide spectral range in a small package. In previous work our group described a spatial heterodyne Raman spectrometer (SHRS) for standoff Raman measurements at visible and UV wavelengths. A standoff spatial heterodyne LIBS spectrometer (SHLS) has not been described. In the case of standoff LIBS measurements the wide field of view of the SHLS design minimizes issues of laser pointing stability and alignment. Also, because spectral resolution is not a strong function of entrance aperture size, high spectral resolution can be achieved in a very small spectrometer using small diffraction gratings. In the described work 10 mm diffraction gratings were used to provide ~0.3 nm spectral resolution. Furthermore, the high light throughput of the system allows LIBS measurements to be made using no collection optics, other than the 10 mm gratings, at distances up to 20 meters. This corresponds to a collection solid angle of less than one microsteradian. In this paper standoff LIBS spectra of minerals and other materials will be shown, collected at distances up to 20 m, without collection optics. Measurements using a small telescope will also be described.
In-situ chemical characterization of deep ocean water around hydrothermal vents requires the development of new classes of multielemental sensors. To this end, laser induced breakdown spectroscopy (LIBS) is being investigated to measure elements in seawater under realistic oceanic pressures, with a long-range goal of deploying a LIBS system on Alvin or other deep-ocean submersibles to measure the elemental composition of deep-ocean hydrothermal vent fluids. In previous work LIBS measurements of alkali and alkaline metals were shown at ppm levels at pressures up to $3 \times 10^7$ Pa (~2800 m water depth equivalent), and it was shown that matrix interactions between different elements were not a major issue. The use of O and H as internal standards was also shown to be promising to correct for emission intensity variations. In more recent work the effect of suspended particulates is being investigated. In one preliminary study using highly absorbing TiO2 particles, the emission intensity for dissolved elements decreases in proportion to the optical density of the solution. Other studies are underway to determine the effect different types of suspended particles will have on the LIBS emission of dissolved elements in seawater. This paper will focus on studies aimed at improving the precision of LIBS measurements in high pressure water using O and H as internal standards, and on studies of the effect of suspended particle loadings for different types of particles on the LIBS emission intensity of dissolved elements.

**Keywords:** Atomic Emission Spectroscopy, Elemental Analysis, Environmental/Water, Instrumentation

**Application Code:** Other

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
The applications of engineered nanoparticles (ENPs) are increasing and widespread. With this increase in applications comes an increase in the environmental exposure of ENPs. As such, it is imperative to understand what happens to them under environmental conditions and if they will persist through environmental treatments such as drinking water treatment processes. Because the expected environmental concentrations of ENPs are low, it is important to develop methods that can detect them at their natural levels in a complex environmental matrix. Single-particle ICP-MS (SP-ICP-MS) provides a new avenue for the detection of nanoparticles in environmental samples based on its super sensitivity and multi-element capacity. The objectives of this study are to develop sensitive, high-throughput SP-ICP-MS methodologies for detecting the presence of both dissolved and nanoparticulate metals present in water and to use these methodologies to study the occurrence and evaluate the fate of ENPs through varied drinking water treatment processes. SP-ICP-MS methods were developed using a NexION 300D ICP-MS system with unique Syngistix\textsuperscript{TM} software developed by PerkinElmer. Screening of ENPs in Missouri drinking water systems was conducted at different seasons. Drinking water treatment simulations were performed by undergoing each major step of treatment – softening, coagulation, filtration, and disinfection. Samples were collected after each step and analyzed by SP-ICP-MS methods developed. The tested ENPs were mostly removed by lime softening and coagulation processes. This project was supported by PerkinElmer and Missouri Department of Natural Resources.

**Keywords:** Elemental Analysis, Environmental Analysis, Method Development, Nanotechnology

**Application Code:** Environmental

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
Atomic Spectroscopy - Instrumentation and Analysis

Generation of Volatile Cadmium Species in Acidic Media Using Al(III), Sc(III), Y(III) and KCN for ICP-MS Measurement

Cadmium (Cd) contamination is closely monitored in water, food and health care products since it is one of the most toxic elements. Vapor generation is an alternative approach for measurement of trace amounts of Cd from complex samples by atomic and mass spectrometry, but it is not often preferred because of the low vapor generation efficiency and substantial inferences from transition metals of sample matrix. In this study, a new vapor generation method has been developed for measurement of low levels of Cd by ICP-MS. The effects and efficiencies of Al(III), Sc(III) and Y(III) were examined for generating volatile cadmium species in the presence of potassium cyanide (KCN). The sample solutions were mixed on-line with 0.16 M KCN and 0.04 to 0.08 M solutions of Al(III), Sc(III) or Y(III) and then reacted with sodium borohydride (NaBH₄). The effect of solution acidity was examined from 0 to 12% HCl. Cadmium vapor formed between 3 to 7% HCl range. Al(III) appeared more effective than Sc(III) and Y(III). Experimental evidence indicated that metal ions act as catalyst in generation of Cd vapor from reaction of acidic samples solutions with NaBH₄. An improvement up to a factor of 10-12 was achieved. The interferences from transitions metals ions were investigated on generation of Cd vapor. The method is validated by analysis seawater samples and various complex samples ranging from purely organic to inorganic by ICP-MS.

Keywords: Environmental/Water, Hydride, ICP-MS, Method Development

Co-Authors: Vedat Yilmaz, Zikri Arslan
Tea is one of the most popular beverages in the world. In recent years, the components in tea leaves have received great concern because they are related to health and disease. The leaves can supply many trace elements essential for human, which have positive effects on health. And different types of tea leaves contain different amount elements, so we can choose the appropriate tea as body needed. So determination of multi-elements in tea leaves is very important. The main objective of this work is to determinate of multi-elements (Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Zn) using ICP-AES, and the Selenium was measured by ICP-AES coupled with HVG-1 (Hydride Vapor Generator). The accuracy of this method was evaluated by measuring the tea standard reference material GBW10016, and the results matched the certified values well. The RSD for every element was less than 3.0%. This work also describes the performance and mechanism of the hydride generator, which can highly improve the sensitivity of Hg, Se, As and so on. This method is rapid and convenient, with high accuracy and good precision, which can be widely used for determination of major and trace elements in the tea leaves.
This paper will focus on assessment of human hair quality with time, based on determination of concentrations of selected metals, e.g. Cr, Zn, Fe, and Cu, in human hair. As aging occurs, the quality of hair decreases. The hypothesis is that degradation of hair quality with time is partly due to concentrations of the aforementioned metals found in the hair. The prediction is that the concentrations of these metals will decrease with time.

At least 60 human hair samples were collected from a wide range of age groups. Each sample was prepared by microwave digestion using nitric acid, followed by dilution of the digests to 25 mL with deionized water. Concentrations of Cr, Zn, Fe, and Cu in the digests were determined by flame atomic absorption spectrometry (FAAS), using an air-acetylene flame.

Experimental procedures, preliminary results obtained, and future directions for this research will be presented and discussed.
Leaching of Lead Through Soil In and Around a Recreational Shooting Range Built on a Reclaimed Strip Mine: Preliminary Results for Lead and Other Analytes

Leaching of lead through soil may be a problem in areas where lead content is high. This problem may be exacerbated by soil with a low pH, such as soil in and around reclaimed strip mines. The determination of lead is especially important, because it is well known that lead is toxic to humans and wildlife. In this specific case, the goal is to investigate whether or not soil acidity exacerbates or alleviates lead leaching. Determination of lead concentrations in, as well as the pH of, soil in and around a recreational shooting range built on a reclaimed strip mine will be performed. The soil at this site will be sampled and analyzed for lead in increments downhill from the range, as the grade slopes toward a manmade lake. In addition to the soil on the range itself, the lake water will also be tested for pH and analyzed for lead levels. Other determinations, such as dissolved oxygen, electrical conductivity, and water hardness will aid in characterization of the overall quality of the water as it relates to metal content in the ground. These determinations will allow for a thorough and guided assessment of the soil and water quality, as both are potentially affected by either the shooting range or the reclaimed mine, or a combination of the two.

Results from the aforementioned determinations, along with details of sample collection, preparation, and analytical methods used, and future directions for this research, will be presented and discussed.

Keywords: Atomic Absorption, Environmental/Soils, Lead, Water
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Molecularly imprinted polymers (MIP) have been used in an increasing number of applications to selective molecular recognition. With this technique is possible to create template-shaped cavities in polymer films during the polymerization process that are complementary in shape, size and chemical functionality to the analyte molecule used as template during the synthesis of polymers. Then the template molecule is removed from the polymer matrix, leaving behind a cavity complementary in shape and size to the template. The obtained cavity can work as a selective binding site for a specific template molecule.

In this work the MIP concept was used to develop enantio-selective electrochemical sensors for rapid, sequential and quantitative determination of L-tyrosine and L-tryptophan. The sensor consists of a gold electrode modified electrochemically with a thin film of polypyrrole in the presence of the amino acids and subsequent over-oxidation of the film. The result is the generation of an overoxidized polypyrrole film with molecular cavities for the highly selective detection of the aminoacids even in the presence of their respective enantiomers.

The results showed that the amperometric sensors have a lineal response (R=0.99) between 0.1-100 mM, with a limit of detection of 6.3 and 9.4 micromolar for L-tyrosine and L-tryptophan respectively. The amperometric response of the sensors was reproducible up to 36 hours when they were stored in a solution containing the corresponding aminoacid. A sensor for the simultaneous detection of the two aminoacids was also studied, the results showed a higher amperometric response for L-tyrosine and with a sensitivity of ca. 70% of the individual sensors.

Keywords: Amino Acids, Electrode Surfaces, Electrodes, Sensors
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Hydrogen sulfide (H2S) is a known toxic industrial pollutant, but also a third endogenous biochemical gaseous signaling compound (after NO and CO). Involvement of hydrogen sulfide (H2S) has been regarded as an essential factor in various physiological processes such as vasodilation, antioxidation, and anti-apoptosis. Recently, it has been demonstrated that the concentration of H2S in Alzheimer’s disease and Down’s syndrome patients’ brains deviates from the normal physiological level, and could be used in diagnostics purposes. H2S’s presence at low (from nanomolar to micromolar) concentrations in biological media has made it a popular target for the development of fluorescent chemosensors. However, none of the described H2S-responsive chemosensors were amplifying, whereas, due to the low concentration in physiological media, its detection would benefit from an intrinsic signal amplification provided by the “higher energy gap” mechanism. In this presentation, we will focus on a water-soluble fluorescent conjugated polymer as an H2S sensor taking advantage of the “higher energy gap” paradigm that was recently developed in our group. Its operating principle is based on an analyte binding to the specially designed receptor chromophore creating a “higher energy gap” site acting as a “roadblock” to effectively limit an intramolecular exciton migration thereby providing an amplified turn-on fluorescent response. The details on preparation of this sensing conjugated polymer, as well as photophysical characterization and H2S detection studies will be reported in this presentation.

The research was funded by the National Science Foundation (CAREER award CHE-0547895 and grant CHE-1362686).

Keywords: Fluorescence, Polymers & Plastics, Sensors
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
The non-enzymatic glucation of albumin has been extensively studied with D-glucose because of the relevance of the sugar to diabetes. Like D-glucose, D-galactose can non-enzymatically react with albumin in vivo in conditions such as galactosemia. The mechanistic similarity in the formation of non-enzymatically glucated and galactated albumin has been the subject of several studies and has generated interest in the characterization of the early and late galactation products of the protein. The objectives of this study were three-fold: 1) to study the advanced glycation endproducts (AGEs) of albumin with D-galactose, 2) to characterize the side chain D-galactose modification of albumin’s AGEs, and 3) to evaluate the effects of galactation on the secondary structure of the protein. AGEs of galactated albumin were evaluated by fluorescence spectrometry at three sets of excitation and emission wavelengths. Excitation/emission wavelengths of 360 nm and 430 nm allowed the measurement of the total fluorescent AGEs. Excitation/emission wavelengths of 335 nm and 395 nm facilitated the measurement of the protein’s pentosidine and argypyrimidine residues. At excitation and emission wavelengths of 485 nm and 530 nm the measurement of arginine modified AGEs of albumin was achieved. CD spectroscopic analysis of galactose modified albumin showed decreases in the helical content of the protein confirming changes in its secondary structure. These findings warrant further investigation as the management of patients with galactosemia has been proposed with galactated albumin.
A Robust Multiple-Aptamer-Based Labeling Method

Since the detection of cancer needs significant accuracy, to get signal from a specific kind of cancer cell without leaking responds from other cells is very crucial. Most of the existing cancer detection methods are analyzing one kind of biomarker at one time, providing less information and efficacy than clinical requirement. Due to the low specificity and selectivity of single-biomarker detection, we designed an aptamer-based multiple-biomarker labeling method for cancer cells detection with enhanced accuracy and specificity. We use two aptamer-terminated oligonucleotides as probes to target two biomarkers respectively. Probe 1 is composed of aptamer sgc8, a T-linker and DNA sequence ab with Cy3 modified on the 3’ end. Probe 2 is composed of aptamer TCO1, a T-linker, strand a’ (the complementary sequence of a) and Cy5 on the 5’ end. A blocker strand a’b’ (the complementary sequence of ab), with two photosensitive PC-linkers embedded in the middle, is used to avoid leaking reaction of Probe 1 and 2 in solution. After incubating with the cell sample, a UV light is used to cleave the blocker, then Probe 1 and 2 will be partially hybridized only if both target biomarkers exist on the cell membrane, leading a FRET between Cy3 and Cy5. With this method, we can accurately detect the cell with two biomarkers, especially can differentiate target from a mix of two kinds of cells with the two biomarkers separately.

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Weihong Tan
Session Title: Bioanalytical Samples - Electrochemical, Fluorescence/Luminescence, Sensors and Lab-on-a-Chip Tech

Abstract Title: Identification of Biological Ligands for Hazelnut Allergen, Cor a 1.02, Using a Fluorescence-Based Assay

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Abstract Text:
As a member of the tree nut family, hazelnut ([i]Corylus avellana[/i]) is included in the Big Eight food allergens. As with other food allergens, avoidance by sensitive patients is the only treatment option; however avoidance of hazelnut is difficult as it is often a “hidden” ingredient in pastries and chocolates. Allergic reactions to hazelnut range in severity, from rare life-threatening anaphylaxis to the more common manifestation of oral allergy syndrome (OAS). Like OAS caused by fruits and other nuts, OAS from hazelnuts is present in patients sensitized by birch pollen ([i]Betula verrucosa[/i]) and is due to the cross-reactivity of homologous proteins in hazelnut and pollen, namely Bet v 1 to several Cor a 1 isoforms. Bet v 1 and Cor a 1 are part of the pathogenesis-related protein family, known as PR10 proteins, which are important for stress response and antimicrobial defense in plants. Bet v 1 and its homologs are characterized by a seven-stranded anti-parallel beta sheet that forms a hydrophobic pocket. This pocket has been shown to bind small hydrophobic compounds, such as steroids, cytokinins and fatty acids. We sought to investigate the ligand-binding ability of Cor a 1 using a fluorescence-based spectroscopic assay. We expressed recombinant Cor a 1.02 in [i]Escherichia coli[/i] and purified it to homogeneity using ammonium sulfate precipitation and ion-exchange and hydrophobic-interaction chromatography. We screened many potential physiologically relevant ligands for binding to rCor a 1.02 by analyzing displacement of a fluorescent ligand, ANS, and found it can bind fatty acids, isoflavones and resveratrol.

Keywords: Chromatography, Fluorescence, Protein, Spectroscopy
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Surface-enhanced Raman spectroscopy (SERS) has been established as a powerful tool for both quantitative and qualitative analyses of environmental samples. When SERS substrates are coupled with appropriate self-assembled monolayers, compounds of interest may partition within the SERS sensing region of the substrate and highly sensitive measurements can be realized. However, fabricating reproducible SERS substrates has remained a challenge, as many methods rely on the randomized close packing of spherical colloids during the fabrication scheme. Here, we have produced a high-performance SERS substrate of aligned silver nanorods using hole-mask colloidal lithography that overcomes these traditional limitations. It can be fabricated over a large area and even patterned into a microarray format. Optimal size of the aligned nanorods was determined through finite-difference time-domain (FDTD) simulations and confirmed empirically. The resulting nanostructures were then applied toward analysis of various perfluorinated compounds, contaminants currently of high concern, within environmental samples.
Five symmetric Schiff base compounds of substituted N,N'-bis(salicylaldehyde)-propylenediamines series were prepared and characterized with electron donating and withdrawing substituents (Methyl, 3', 4' and Difluoro) on the benzene ring and tested for their antimicrobial activity. The synthesized compounds were characterized by elemental analysis, FTIR and 1H, 13C, and 19F NMR. Spectrophotometric titration of these compounds was performed in the pH range of 11.00 to 4.00 in aqueous solutions. Using the absorbance intensity (370 – 390 nm peak) variations with changes in pH, the relative pKa values of the protonation of the nitrogen atom of the Schiff’s bases were determined. The experimentally determined values of pKa by sigmoidal non-linear regression of the pH vs absorbance for the Schiff bases ranged from pKa = 6.3 for the N,N'-bis(3'-fluorosalicylaldehyde)-propylenediamine to pKa = 7.70 for N,N'-bis(5'-fluorosalicylaldehyde)-propylenediamine. Electron donating methyl substituent on the phenyl ring, in the compound N,N'bis(3'methyl salicylaldehyde) propyleneamidine is found to make the compound less acidic; pKa = 8.51. The enhanced acidity of fluoride substituted species relative to N,N'-bis(salicylaldehyde)-propylenediamine, pKa = 8.11, is attributed to the inductively electron withdrawing fluorine on the benzene ring favoring the Keto-amine form of the Schiff’s base, (250 nm peak of cyclohexadienone at 3.5 pH in the UV-Vis spectra), over the Enol-imine form. Antimicrobial activity of these compounds were measured against Escherichia coli, Bacillus subtilis, and Pseudomonas fluorescens Pf-01 using standard minimum inhibitory concentration assays (MIC) and bactericidal assays at >500 µM concentration. The activity of these compounds suggests that the difluoro ligand is capable of inhibiting growth at >500 µM but is not bactericidal at the same concentration. In addition, gram negative bacteria are the most sensitive to this class of compounds.

Keywords: Bioanalytical, Pharmaceutical, Spectrophotometry, UV-VIS Absorbance/Luminescence
Application Code: Bioanalytical
Methodology Code: UV/VIS
The electrochemical proximity assay (ECPA) is a direct-readout proximity immunoassay with femtomolar limits of detection for proteins, and we have shown this technique to be capable of direct quantitation of hormone secretion from small numbers of endocrine cells. However, ECPA relies on DNA annealing at the surface of a gold electrode, which is exceptionally sensitive to temperature fluctuations. To further stabilize ECPA readout, a custom-tailored thermal regulator was created to control temperature between 0 to 100 °C. The system was designed utilizing an integrated controller for peltier modules, capable of delivering 2.2 amperes at up to 5 volts. A 30x30 mm peltier module capable of sustaining a 66 °C temperature difference was used for the thermal pumping. The hot side of the peltier module was fitted with a 40x40x23 mm heat sink and a 30x30 mm fan capable of 3.72 cubic feet per minute of airflow. The controller was driven with an in-house written LabVIEW application. The program integrates safety shutdown procedures, proportional integral derivative control, and data capture functionality. The system's resolution is ±1 °C in the experimental setup over the course of a 2 hour run. To evaluate the system, thermal melting analysis was carried out on a DNA loop model of ECPA. A 7 base-pair DNA strand with a methylene blue label (electrochemically active) was used to model background, and a DNA loop was added to model signal. Thermal melting analysis with our system showed melting of the signal strands at 30 °C, and melting of the DNA anchor strand at 48 °C. With this novel control system, it should be possible to thermally resolve signal and background in ECPA and significantly improve its performance for direct protein quantitation.

Keywords: Bioanalytical, Biosensors, Electrochemistry, Sensors
Application Code: Bioanalytical
Methodology Code: Sensors
The aim of this paper is on the development of the biosensor platform capable to explore the biocatalytically initiated polymerization process. Such a platform will enable the combination of the biocatalytic process, the chemical amplification, and the plasmonic effect in one device. The ability to sequentially combine differing initiation mechanisms with this coating approach was achieved by using glucose oxidase mediated interfacial polymerization on hydrogel substrates initially polymerized in the presence of glucose and fenton reagent. This approach can be potentially applied to many different biosensing schemes based on enzymes capable of metabolizing target biological analytes in a complex environment.

Keywords: Biosensors, Materials Science, Nanotechnology

Abstract Text

Date: Monday, March 09, 2015 - Morning
Room: Exposition Floor, Hall F, Aisles 390
Phosphatidic acid phosphatase, PAP (EC 3.1.3.4), is a committed step in triacylglycerol (TAG) synthesis which is also known as Kennedy pathway. The enzyme catalyzes the penultimate step in TAG synthesis to form Diacylglycerol (DAG) which is then acylated to form TAG by diacylglycerol acyltransferase (DAG). PAP essentially controls the pool of DAG and controls how much TAG will be synthesized. Typically, PAP activity is measured by radiolabeled phosphatidic acid, which is costly and cumbersome method. We have developed colorimetric methods to measure PAP activity using dioleoyl phosphatidic acid (DPA). In this paper, we presented evidence that DPA could be substituted by β-glycerophosphate (BGP). We also performed binding experiments with biosensors using immobilized or bound DPA and BGP onto aminopropylsilane activated sensor in a BLITZ system. In both cases, the biosensors showed binding (association) and release (dissociation) from the biosensor. Taking both the enzymatic and binding data, we conclude that BGP could substitute DPA to drive down the PAP activity. These findings pave the way for BGP to substitute DPA or other phosphatidic acid whose solubility in biological buffers is an issue. Enzymologists now could perform PAP activity using nonradioactive substrate cost effectively without having the issue of adding detergent to the enzymatic mix for greater solubility of phosphatidic acid.
Copper is the first and only metal to be recognized by the U.S. Environmental Protection Agency as having anti-microbial properties. In the aquarium industry, copper has long been used as an anti-algae agent by home aquarists and as a treatment for the protozoan parasite [i]Cryptocaryon irritans[/i] a.k.a. Marine “Ich.” Therapeutic levels for Ich are ca. 150 to 200 ppb; however, at concentrations greater than 220 ppb the marine life is threatened from a reduced immune response. For the aquarium industry, there is a pressing need for an analytical method that can analyze copper on the therapeutic scale, [i]in situ[/i], with a minimum of analysis time.

While the literature is replete with methods for the analysis of copper, most of these methods are either not capable of [i]in situ[/i] analysis or have limits of detection and quantification that are far above the therapeutic limit for copper in marine systems. Pulsed electrochemical detection (PED) of copper allows for a combination of the deposition and stripping of anodic stripping voltammetry coupled to the signal enhancement of a near-infinite scan rate offered through chrono-amperometry.

This research focuses on development of a PED waveform for the analysis of copper in marine solutions with focus on the development of an [i]in situ[/i] sensor. Initial work was done utilizing Cyclic Voltammetry to obtain qualitative information on the Cu2+/Cu0 reduction-oxidation couple in the marine environment. From this data an initial waveform was developed and optimized, with emphasis being placed on having the shortest possible total time for rapid cycling. Limits of detection and quantification were obtained using the standard additions method. The sensor was validated using a blind study. Artificial seawater was obtained from the National Aquarium (Baltimore, MD) and all work was done on a glassy carbon rotating disk electrode to ensure that marine components would not react with the electrode surface.

Keywords: Bioanalytical, Environmental/Water, Metals, Stripping Analysis
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Bioanalytical Samples - Electrochemical, Fluorescence/Luminescence, Sensors and Lab-on-a-Chip Tech

Detection of PCR Amplified DNA from [i]Neisseria Gonorrhoeae[/i] and [i]Chlamydia Trachomatis[/i] on a Disposable Lateral Flow Strip

[i]Neisseria gonorrhoeae[/i] and [i]Chlamydia trachomatis[/i] are the two most prevalent bacterial sexually transmitted infections (STIs) reported to the Centers for Disease Control and Prevention (CDC). Since these infections, especially [i]C. trachomatis[/i], are most often asymptomatic they are usually not diagnosed early. Traditional laboratory diagnosis of these infections is done by culture for [i]N. gonorrhoeae[/i] and cell culture or antigen detection for [i]C. trachomatis[/i]. Recently, Nucleic acid amplification tests (NAATs) have become widely accepted and are now recommended by the CDC as the test of choice; however current NAATs are classified as high or moderate complexity and may take 1-2 days for results to become available. New assays and new platforms which provide results at the time of patient visits are urgently needed, since many patients do not return for their results when laboratory-based tests that require several days are performed. We report a quick DNA-AuNP based lateral flow nucleic acid biosensor for the sensitive detection of multiple DNA targets based on sandwich hybridization reaction. Accumulation of AuNP conjugates on the test zones enabled visual detection of nucleic acids sequences. A detection limit of 0.1nM was achieved by measuring the intensity of the red zones via a portable strip reader. Simultaneous detection of DNA from [i]Neisseria gonorrhoeae[/i] and [i]Chlamydia trachomatis[/i] strain samples was performed using this concept.

Keywords: Biosensors, Detection, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip

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## Intracellular Superoxide Detection Using Microchip Electrophoresis Coupled to Laser Induced Fluorescence Detection

Reactive oxygen species (ROS) are part of the natural aerobic metabolism of cells and are involved in various signaling and regulation processes. However, ROS can also cause oxidative stress when antioxidant defenses of the cell fail to regulate their production. Specifically, superoxide can cause cellular oxidative stress through its dismutation to hydrogen peroxide and molecular oxygen as well as by reacting with nitric oxide to produce peroxynitrite, an extremely toxic molecule. However, due to its high reactive nature, superoxide can be difficult to quantitate. Therefore, the present work focuses on a method for the quantitation of superoxide produced inside RAW 264.7 macrophage cells before and after external stimuli.

MitoSOX is a mitochondria targeted fluorescent probe that produces a specific one-electron oxidation product ($2\text{-OH-MitoE}^+[/sup]$) upon reaction with superoxide and was used in these studies. The reaction of MitoSOX with other intracellular hydride acceptors can generate a different two-electron oxidation product. In order to separate the different reaction products, microchip electrophoresis was elected since it has been used to analyze bulk cell lysates and can be configured later for single cell analysis studies on cell heterogeneity. The $2\text{-OH-MitoE}^+[/sup]$ standard was obtained by the reaction of the probe with nitrosodisulfonate (NSD). The identity of the peak was confirmed by comparing migration times with the product formed in a xanthine/xanthine oxidase system. Finally, macrophages were incubated with the probe after stimulation and the results compared to non-stimulated samples.

### Session Title
Bioanalytical Samples - Electrochemical, Fluorescence/Luminescence, Sensors and Lab-on-a-Chip Tech

### Abstract Title
Intracellular Superoxide Detection Using Microchip Electrophoresis Coupled to Laser Induced Fluorescence Detection

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### Abstract Text
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### Keywords
- Bioanalytical
- Fluorescence
- Lab-on-a-Chip/Microfluidics

### Application Code
- Bioanalytical

### Methodology Code
- Microfluidics/Lab-on-a-Chip
Abstract Text

In this work, we present a rapid and highly sensitive approach for visual detection of microRNA (miRNA) using gold nanoparticles (GNP) coated silica nanorod (SiNR) label and lateral flow strip biosensor (LFSB). GNP were decorated on the SiNR surface by a seeding and growth procedure. Single strand DNA probe was immobilized on the GNP-SiNR surface by a self-assembling process, and the formed DNA-GNP-SiNP conjugate was used to construct the LFSB for detecting miRNA. The captured GNP-SiNR by sandwich-type hybridization reactions (DNA-RNA-DNA) on the test zone of LFSB produced the characteristic color bands, enabling visual detection of miRNA. The miRNA-215 was used as a model target to demonstrate the proof-of-concept. After systematic optimization, the new LFSB was capable of detecting 10 pM of the miRNA target without instrumentation, which is 6 times lower than that obtained with the GNP-based LFSB. Such significant signal enhancement with the GNP-SiNR label is due to the increased number of GNP per DNA-RNA hybridization event. The GNP-SiNR thus provides a new and sensitive nanolabel for visual detection of biological molecules on the lateral flow biosensors, and shows great promise in clinical application and biomedical miRNAs diagnosis in some malignant diseases.

Keywords: Biosensors, Lab-on-a-Chip/Microfluidics, Nanotechnology, Portable Instruments
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
An Integrated “SERS Spectrometer” based on Evanescent Field Excitation

We will introduce a new design of SERS analyser, which is a new SERS excitation and detection setup based on the evanescent field excitation. This SERS analyser has a special optorode which integrates a solid immersion lens and a SERS-active substrate, allowing for the directional excitation and emission of SERS exactly at the resonance angle. Different from the commercial and other-made “Raman spectrometers”, this SERS spectrometer integrates a metal film with enhancement activity. Its sensitivity is above the commercial Raman spectrometer and it will be promising in surface/interface SERS analysis and medical diagnosis.

This work was supported by National Instrumentation Program (NIP) of the Ministry of Science and Technology of China No. 2011YQ03012408, the National Natural Science Foundation of China NSFC Grant Nos. 21373096, 21073073 and 91027010 and Innovation Program of the State Key Laboratory of Supramolecular Structure and Materials.

Reference
1. China Patents for invention, filling No. 2011101758766.

Keywords: Raman, Spectrometer
Application Code: Bioanalytical
Methodology Code: Portable Instruments
A major difficulty encountered in crime scenes is the ability to differentiate human blood from animal blood. Current testing kits for differentiating human from animal blood have several shortcomings. For example, in addition to being costly, they typically rely on time-consuming protocols and require the use of monoclonal antibodies. The purpose of this research project was to explore the utility of FT-IR technology for the development of a rapid, accurate, and user-friendly method for discriminating human blood from animal blood; a test aimed at overcoming many of the difficulties and limitations of current testing procedures. To compare the FT-IR spectra of human blood with animal blood two types of analyses were performed. The first technique relied on the evaluation of spectra by standard classic spectroscopic methods. The second method relied on the use of Support Vector Machine (SVM) algorithm which offered a unique multivariate approach for the unbiased analysis of the data. The human samples used in this study included 107 blood specimens of which 35 were typed as blood group A, 26 as blood group B, and 21 and 25 as blood groups AB and O, respectively. The other blood specimens were obtained from animals of different sizes such as cats, dogs, horses, and mice with five samples taken from each of the animal species. Classic spectroscopic studies combined with SVM demonstrated distinct differences between human and animal FT-IR spectra, and verified the potential of the FT-IR method in a forensic setting, warranting further exploration of this technique.

Keywords: Biological Samples, Forensic Chemistry, FTIR
Application Code: Bioanalytical
Methodology Code: Near Infrared
Objective: The objective of this study was to determine the effects of fructose and their advanced glycation end products (fru-AGES) on histone acetylation in microglia, the immune cells of the brain.

Significance: Fru-AGES primarily form as a result of non-enzymatic reactions between fructose and proteins. One result is inflammation in the brain, which can be directly correlated to increased microglia activity. Microglial activity has been shown to be associated with the acetylation of histones, resulting in a change in transcription of inflammatory genes. Elucidation of a direct link between fructose, fru-AGES and histone acetylation would increase understanding the pathophysiology of inflammatory disorders such as Alzheimer’s disease.

Experimental Procedures: An immortalized rat microglial cell line was treated in vitro with control media, fru-AGES or fructose. Histone acetylation was analyzed indirectly through activity of histone deacetylase (HDAC) using the HDAC Glo I/II Assay (Promega). Chemiluminescent product formation was measuring with a spectrophotometer.

Results Obtained: Both treatments with fructose and fru-AGES showed an increase in HDAC activity compared to control by up to 35% and 20%, respectively, correlating to a decrease in global histone acetylation. This is contradictory to initial expectations, as a decrease in acetylation could result in a decrease in transcription of genes. Despite causing an initial inflammatory response, fructose and fru-AGES appear to suppress overall gene transcription.

Conclusion: Previous data show that exposure of microglia to fructose and fru-AGES results in a pro-inflammatory activated state. However, at the level of gene transcription, microglia may be desensitized and less able to respond in the long term.

Keywords: Genomics, Neurochemistry, Spectrophotometry, Toxicology
Application Code: Neurochemistry
Methodology Code: Fluorescence/Luminescence
Preterm birth (PTB) is a birth occurring prior to 37 weeks of pregnancy; it is the most common type of complication in pregnancy, leading to neonatal deaths and newborn illnesses. At present, effective clinical methods that can assess the risk of PTB at a stage where therapeutic interventions are still possible to delay delivery are not readily available. Thus, there is a clear need for a simple, cost-effective and rapid analysis system for detection of PTBs. Esplin et al. previously discovered three maternal serum peptides, which when combined with six previously characterized protein biomarkers, provided 87% sensitivity and 81% specificity in predicting a PTB four weeks later at a gestational age of 28 weeks.1 We are developing an integrated microfluidic platform that can extract, label, separate and quantify these PTB biomarkers directly from blood samples, to address the above mentioned need. We are optimizing individual modules of this integrated platform in a parallel fashion. We have successfully carried out microchip electrophoresis separation of several PTB biomarkers. We are also characterizing reversed-phase and immunoaffinity monolithic columns for on-chip labeling and immunoaffinity extraction of PTB biomarkers. Our ultimate goal is to integrate multiple processes, including immunoaffinity extraction, solid-phase sample enrichment, on-chip labeling, electrophoretic separation and quantification, on a single microdevice. We believe that the utility of this novel platform will extend well beyond PTB biomarker analysis to various other prospective analytes, therefore offering great potential for improving human health.

Reference:

Keywords: Analysis, Electrophoresis, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
In Vivo Electrochemical Measurements of Optogenetic-Stimulated Release of Single Varicosities in Drosophila Melanogaster Larvae

This work faces the challenge to electrochemically measure neurotransmitter release from the smallest varicosities of a nerve cell. The nerve terminals found in the body wall of Drosophila larvae are readily accessible to experimental manipulation. We used the light-activated ion channel channelrhodopsin-2 (ChR2), which is expressed genetically in type II varicosities, to study octopamine release in Drosophila larvae. Here we report the development of amperometry to measure release of the neurotransmitter octopamine from individual varicosities in the Drosophila larval system by amperometry. A carbon-fiber disk microelectrode was placed onto this cellular region of the muscle and held at a potential above the formal oxidation potential of octopamine and the terminal stimulated by blue light. The results suggest that optical stimulation can be used to evoke exocytosis release in the type II varicosities of Drosophila larvae. When octopamine is released from the varicosities during stimulation, it is oxidized at the electrode and a current spike results. We use this method to quantify the amount of transmitter released and these subsecond release events. This is a new approach to study millisecond release events in the nanoliter environment of a neuromuscular junction.

Keywords: Analysis, Electrochemistry, Electrodes, Neurochemistry

Application Code: Neurochemistry

Methodology Code: Electrochemistry
Within the central nervous system, histamine (HA) is involved in numerous brain functions including awakening, the control of pituitary hormone secretion, and cognitive functions. In recent studies, altered levels of HA has been speculated to be involved in brain diseases such as Alzheimer’s, Parkinson’s, and depression. While the general HA pathway originating from the cell bodies in the tuberomammillary nucleus (TM) is generally understood, its’ similar electrochemical signature to other compounds such as adenosine and hydrogen peroxide has made it difficult to characterize in vivo. Using an optimized histamine-sensitive waveform with fast-scan cyclic voltammetry (FSCV) has now enabled us to better understand the innervations of HA within the mouse brain. In this work we utilize our novel waveform and FSCV at highly sensitive carbon fiber micro electrodes in combination with pharmacological analysis to measure HA levels in the substantial nigra reticular (SNr), cerebral cortex (CX), and ventral pallidum (VP) via stimulation of the HA cell bodies in the tuberomammillary nucleus (TM). We demonstrate that robust in vivo detection of HA using FSCV can be used to effectively study, characterize, and implicate the identification of a precise chemical profile of HA in future studies.

Keywords: Electrochemistry, Electrodes, Neurochemistry, Voltammetry

Application Code: Neurochemistry

Methodology Code: Electrochemistry
Chemical cytometry is a form of single-cell analysis in which an individual cell is lysed, and its contents separated and detected. Combined with fluorescently-labeled reporter substrates, chemical cytometry can yield information about cell-to-cell variation in enzyme activity. To date, such experiments have been performed primarily on mammalian cells. However, cellular heterogeneity plays an important role in the biology of many species, including important non-mammalian model systems, such as Dictyostelium discoideum. Application of established chemical cytometry assays optimized for human cells to new model organisms requires thoughtful adaptation of each assay step. We report on our work to apply a peptide reporter substrate for protein kinase B to Dictyostelium cells. PKB is highly conserved threonine kinase involved in cell proliferation and survival in human cells and in chemotaxis during Dictyostelium development. Using fluorescence microscopy, we show that methods used to load reporter peptides in mammalian cells, including pinocytosis with osmotic lysis, electroporation, and myristoylation, yield varying results in Dictyostelium, due to differences in its cellular physiology. We are evaluating the processing of the peptidase-resistant reporter substrate in Dictyostelium lysates using electrophoretic separations in capillaries and microchips. This adaptation of a tool developed in human cell lines will open new possibilities for studying cellular heterogeneity in diverse cell types.
The electrochemical proximity assay (ECPA) is a powerful technique that is capable of detecting proteins at femtomolar levels. ECPA has a wide variety of potential applications, including medical diagnostics and point-of-care testing. In ECPA, formation of a surface bound complex composed of the target protein and two DNA-antibody capture probes brings an electrochemical reporter molecule close to the electrode surface where it can be detected amperometrically. Compared to conventional ELISAs, ECPA has three advantages: lower detection limits, wider dynamic range, and shorter analysis time.

Bipolar electrochemistry has received increasing attention in recent years because of its ease of integration with lab-on-a-chip technology and its wireless readout capability. The marriage of bipolar electrochemistry and ECPA offers a new way to measure protein levels quantitatively without the need for direct electrical connection. In this study, electrogenerated chemiluminescence (ECL) was used to report on the state of the bipolar ECPA sensor. Because of the extremely low currents flowing through the bipolar electrode, we developed a signal averaging strategy in which a time dependent square wave potential program was applied across the bipolar electrode, allowing the S/N of the ECL signals to be enhanced.

Keywords: Bioanalytical, Biosensors, Chemiluminescence, Electrochemistry

Abstract Text
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Three dimensional (3-D) printing technology has evolved dramatically in the last few years, offering the capability of printing objects with a variety of materials, from rubber to more rigid and transparent plastics. Printing microfluidic devices using this technology offers various advantages such as ease and uniformity of fabrication, file sharing between laboratories, and increased device to device reproducibility. One of the most unique aspects of this technology, when used with electrochemical detection, is the ability to produce a microfluidic device as one unit, furthermore allowing the reuse of the device and electrode for multiple analyses. Here we report microfluidic devices printed using two different printing methods, Fused Deposition Modeling (FDM) and PolyJet, which can be used with electrochemical detection. In both cases, we compare the fabrication process of the printing process, materials used for printing- acrylonitrile butadiene styrene (FDM method) and FullCure 720 (PolyJet method), the device design, and the results of the electrochemical detection of various concentrations of catechol. For the design of the devices, we report a comparison between thin layer electrode and wall-jet electrode system. The later system enhances analytical performance by eliminating any dead volume created at the electrode-solution interphase, increasing peak symmetry and reducing tailing. We further discuss the fabrication of custom fittings that houses the electrodes and sample inlet capillaries. Due to the varying costs, resolution capabilities and differences in materials of 3-D printers, this study provides a platform to verify versatility, reproducibility and convenient nature of this technology to produce microfluidic devices.
Microfluidic mixing system has shown considerable efficiency for miniaturizing chemical and biological processes such as stimulation and modification, because of its advantages of small sample consumption, rapid sample processing, precise control of the fluids and high throughput capability. However, such mixing system combines with the substrate limits the target substrate and sometimes suffers from the interference between the microfluidic mixing system and macroscopic solid objects. Here, we have developed a microfluidic mixing chemical pen (MMCP) by combining microfluidics technology and fountain pen, which overcomes those limitations and permits region selection and free movement. The MMCP was easily made by put three capillaries into one large glass tube, which could be fabricated with good reproducibility without high cost. Two of the three capillaries were used for injection of two individual solutions and the third one was used for aspiration. Flow streams underneath the MMCP have stable boundaries under the hydrodynamic forces which act as the walls of microchannels; mixing area similar to microfluidic mixing in closed microchannels forms because of diffusion effect. Thus, the MMCP is freely moved and can easily acts on most types of solid surfaces by moving over them. We evaluate the multifunction of this concept with several models including online protein labeling, modification, protein arraying, and Ag electrode. The MMCP allows region selection and designed scanning by moving over the sample surface.
Abstract Text

Paper-based microfluidic devices (µPADs) are capable of achieving rapid qualitative and quantitative measurements of a variety of analytes inexpensively. µPADs relies on patterning of hydrophilic-hydrophobic regions on a sheet of paper in order to create capillary channels withing impermeable fluidic brakes on the paper. Here we present a novel, highly flexible and low-cost fabrication method using a desktop digital craft plotter/cutter and technical drawing pens with tip size of 0.5 and 1.0 mm. The pens were used with either commercial black permanent ink for drawing fluidic brakes, or with specialty in-house formulated aqueous inks. With the permanent marker ink it was possible to create barriers on paper rapidly and in a variety of designs in a highly flexible manner using a software controlled plotter/cutter - for instance, a design featuring eight reservoirs (Fig.1) can be produced within 10 s for each µPAD. Also, using this method a reproducible line width of brakes can be achieved with %RSD < 1.5. To verify the method flexibility and performance, we further investigated the optimal viscosity range of in-house formulated inks, with a model example of aqueous solution of bromophenol blue (BPB) with the addition of poly(ethylene glycol) in order to vary the viscosity. We show that the drawing pen technique offers a favourable freedom of ink composition compared to ink-jet printing. A function test of these µPADs was conducted by the determination of bovine serum albumin using BPB to form color complex. Lines and areas were plotted on the paper and ImageJ was used to convert the images to gray scale format before measuring the intensity of the colored area. The fabrication method using technical drawing pens provides flexibility in the used inks, short fabrication time, simplicity and low cost.

Keywords: Lab-on-a-Chip/Microfluidics, Portable Instruments
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
We have previously shown that passively operated, polydimethylsiloxane (PDMS) microdevices allow secretion sampling from individual primary murine islets. However, fabrication of the fluidic reservoirs was time consuming and tedious, using custom-made inserts that often shifted alignment or fell over into the uncured PDMS during casting. Here, we show that 3D-printed templates allow interfacing to monolithic, microfluidic channels, permitting 3D ‘landscaping’ features not previously attainable with standard well punching methods. The insert defines a central well with a large reservoir region (7.5 mm) and a smaller (1 mm) well is punched in the bottom of the reservoir (see attached image). 3D modeling and printing of interface templates gives exquisite control over fluidic interfacing designs and drastically reduces batch-to-batch variability. Additional alignment and stabilizing elements were incorporated into the insert fabrication to increase their ease of use. The completed device is capable of secretion sampling from primary pancreatic islet cells loaded into the smaller central inlet. Vacuum is passively applied to each of the 8-channels over alternating time periods to collect temporally resolved samples from the islets in the central well. By flowing out into discrete wells, samples can be easily collected over varying periods of time without having to incorporate more complicated sampling methods. The large/small reservoir interface at the inlet is vital for rapid switching of stimulatory solutions without disturbing cells in the inlet and for reducing dead volume. The device will be further characterized for flow rate and channel resistances using methods previously established in our lab. After initial characterization and proof-of-concept for this device, the subsequent aim is to design a simple, in-house software program with multiple solenoid valves controlling each of the 8-channels to completely automate sampling.

Keywords: Bioanalytical, Biological Samples, Biosensors, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
The pH measurement by commercialized pH paper is a confusing work because the value is arbitrarily determined based on the color changes of several pH detection zones. In this work, a new paper-based pH-measuring device (pH scale bar paper), which shape is similar to a common mercury thermometer, was developed for providing more user-friendly pH paper with original advantages such as short detection time, simplicity and low cost. The pH scale bar paper device in size of 25 x 75 mm² (see Fig. 1A) has a 5-layered structure with PET/adhesive/patterned paper/adhesive/PET, and was fabricated using wax printing, paper cutting by laser, and layer bonding by pressing. It has multiple colorimetric detection zones which respond to specific pH values with different pH indicators, and a sample solution is sequentially delivered from an inlet to the detection zones through a hallow paper channel very fast. Fig. 1B show a prototype device with a resolution of pH = 1, indicating a successful demonstration of the proof-of-concept of this new pH-measuring paper device for a test solution (pH = 13). The analysis time was less than 30 s with the use of plasma-treated hydrophilic PET layers. The color of detection zones was changed sequentially from pH = 1 to 14 (bottom to top), and initial colors of white or light yellow were converted to dark colors if a sample had equal or higher pH than a designed value.
New paper-based fluidic devices were studied for a use in liquid chromatography. A basic concept of the lab-on-a-paper (LOP) chromatography device is to separate analyte molecules under a constant flow rate condition along a stripe paper channel unlike the conventional chromatography in which the flow rate was governed by Lucas-Washburn equation: flow distance is proportional to square root of time. For achieving the quasi-stationary flow condition, our new fluidic device used a fan-shaped absorption pad-linked strip channel. The paper channels with different fan angles were fabricated by wax-printing, and its top and bottom were covered by PET layers to prevent eluent evaporation from a paper channel. From travel distance measurements of flow front, the aqueous dye solution at first moved along the strip channel under the Washburn relationship, while the channel flow rate displayed a nearly constant flow speed after the front reached the fan region. Furthermore, quasi-stationary flow became fast by increasing the fan angle, and the rate was controlled in the region of $1.4 - 2.5 \mu\text{L/min}$, demonstrating that the absorption pad functioned as a paper pump. Our LOP chip give a chance to use a long strip channel in paper chromatography. As a preliminary test, green food dye was injected into the strip channel after developing a quasi-stationary flow, and then successfully separated into yellow and blue components.
Both Polystyrene (PS) and polydimethylsiloxane (PDMS) are useful substrates for the development of chip-based in vitro mimics. A recurring issue with totally integrated devices is that if any part of the chip fails (delamination or cells dislodging), the entire experiment is compromised. The overall goal of this work is the development of a device that can integrate a substrate containing immobilized cells with electrophoresis for analysis of cell releasates. The hybrid device in this work takes advantage of the benefits of PDMS (ability to incorporate peristaltic pumps and valves) and PS (ability to embed fluidic tubing and electrodes). A Pd decoupler is used to ground the system and allow in-channel electrochemical detection for close to real-time analysis. Pneumatic valves isolate cells from electrophoretic voltages. Upon injection, analytes are separated via microchip electrophoresis and detected. Adhesion between substrates was improved with a stamping technique using chlorotrimethylsilane. The separation of dopamine and norepinephrine was optimized in highly conductive biological buffers, and this device was used to monitor the neurotransmitter release from PC 12 cells. It will also be shown that separation efficiency can be improved by lengthening the electrophoresis channel with embedded fused silica capillary. The valving chip makes injections that traverse down the channel, into a capillary loop, and back onto microchip for detection. The embedded capillary can also be used to make devices more modular in a “plug and play” manner, where a cell culture chip can be connected to an analysis chip when desired for a chip-to-chip interface.
Diabetes is a metabolic disease characterized by a hyperglycemic state, which, if not effectively managed, can generate reactive oxygen species (ROS). When ROS attack endothelial cells (ECs), which line the walls of blood vessels, cardiovascular damage develops over time. Despite the strong vascular component of the disease, a suitable \textit{in vitro} model that reflects the consequences of hyperglycemia on cell metabolism \textit{in vivo} has yet to be developed. Conventional approaches to studying diabetes \textit{in vitro} typically involve culturing ECs in cell culture flasks and subjecting them to elevated glucose levels. Because ECs \textit{in vivo} experience constant shear stress as a result of blood flow, such conventional approaches are inadequate as biomimetic models of the disease. The project proposed here describes a novel method of culturing endothelial cells for several days in a microfluidic device consisting of channels fabricated in a poly(dimethylsiloxane) microchip sealed over a glass substrate. Inlets and outlets on the chip allow for flow-based experiments in which cells are continuously provided a supply of fresh media or serum. Microchannel dimensions are consistent with those of a blood vessel and the small fingerprint of the device enables multiplexing while generating little waste. This device has the potential to provide a biomimetic model of diabetes which could be coupled to a wide range of metabolic analysis strategies. This work will investigate the metabolic pathways that become damaged by hyperglycemia which will aid in the elucidation of potential therapeutic targets.

**Keywords:** Bioanalytical, Lab-on-a-Chip/Microfluidics, Liquid Chromatography/Mass Spectroscopy, Metabolomic

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
The separation of biological samples is crucial in the field of bioanalytical chemistry. Two-dimensional separations are becoming increasingly important due to the complexity of modern samples, requiring peak capacities that exceed what can be achieved by traditional one-dimensional methods. Current two-dimensional separations, however, are limited by the under-sampling of peaks as they elute from the first dimension, necessitating frequent sampling of the analyte peaks. To address this challenge, we propose the utilization of micro free flow electrophoresis (µFFE) as a second-dimensional separation technique. In µFFE, analytes are continuously streamed into a planar separation channel while an electric field is applied perpendicularly, allowing for lateral deflection based on electrophoretic mobilities. This continuous separation technique eliminates complicated injection interfaces, maximizing the sampling of peaks as they elute from the first dimension. Consequently, the effective sampling rate is limited only by the exposure time of the camera (typically 100 msec), offering a significant advantage over traditional methods.

To demonstrate the potential of µFFE as a two-dimensional separation tool, we will couple capillary electrophoresis (CE) with µFFE to analyze a tryptic digest of bovine serum albumin. This combination is expected to achieve peak capacities greater than 3000 in less than 15 minutes, showcasing the capabilities of µFFE as a high-speed second-dimensional separation technique.

This work was supported by NSF-CHE Grant #1152022.

Keywords: Capillary Electrophoresis, Electrophoresis, Lab-on-a-Chip/Microfluidics, Other Hyphenated Technique
Application Code: High-Throughput Chemical Analysis
Methodology Code: Microfluidics/Lab-on-a-Chip
Abstract Text

This abstract reports an electrokinetic manipulation to separate polystyrene (PS) microspheres in high-conductivity media, although most electrokinetic operation only focusing in low-conductivity buffers. A PDMS/glass microfluidic chip with a designed of a three-layer sandwich, and three-parallel micro-electrode was fabricated for the different size of PS microspheres separation. With optimized AC voltage and frequency, we demonstrate that 25 μm microspheres can be isolated from 5 or/and 10 μm microspheres and concentrated onto the inner electrode in dozen sec. The design of the microfluidic chip is shown in Fig.1. The microchannel was fabricated by molding PDMS with laser machined mold. The electrodes were fabricated by sputtering Ti-Au-Ti on a glass slide. Then the glass substrate with microelectrodes was sealed with the PDMS microchannel by plasma treatment. There three sizes of PS microspheres (5, 10 and 25 μm respectively) were used which were evenly dispersed into the conductive solution (10X TAE, ~1.59 S/m) before experiment. To separate two or three sizes of microspheres, a series of electric field were applied. It was visible that, the 5, 10 and 25 μm microspheres were positioned into different equilibrium locations. In particular, almost all of the 25 μm microspheres migrated to the inner electrode, while the majority of 5 and/or 10 μm microspheres moved into the areas between the neighboring electrodes (Fig.2). Fig.3 shows that the results of the separation, in which, at the frequency range of 100 kHz-10 MHz, 5 and 25 μm, 10 and 25 μm PS microspheres can be separated. By optimized frequency and AC voltage, we demonstrated that the better of frequency for the separation of two sizes of microspheres (10 and 25 μm, 5 and 25 μm) (fig.4) and three sizes of microspheres (5, 10 and 25 μm) were 100 kHz, 2 MHz and 1 MHz separately. Meanwhile, the better AC voltage conditions for two or three sizes of microspheres separation were 14 V, 10V and 11V respectively.

Keywords: Biosensors, Electrodes, Lab-on-a-Chip/Microfluidics, Separation Sciences
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Exocytosis is an essential cellular process in neuronal communication. This cellular function involves vesicle fusion and vesicle content release of neurotransmitter molecules. In the quest to determine the molecules affecting exocytosis, simplified model systems, such as artificial cells are valuable tools. Model systems allow us to study the effect of different components such as various lipids on the process of exocytosis in a controlled manner. Membrane lipids including cholesterol provide the platform for carrying out and regulating exocytosis. Although cholesterol is a major component of the membrane and certainly affects exocytosis, the overall biochemical and biophysical properties have not been fully understood. The goal of this work is to use an artificial cell model and amperometry to provide information to better understand the effect of cholesterol on exocytosis. In this work we are using two different artificial cell models: one cell model based on pure lipid composition and a second cell model constructed from cell plasma membrane from a PC12 cells. Kinetic information of single vesicle release of dopamine is recorded using carbon fiber amperometry and provides information that will help to better understand the effect of cholesterol on exocytosis. The two cell models used in this work differ in the overall membrane tension, a parameter that we show with the bleb cell model can influence whether the fusion pore fully or partially distends. Hence, both partial and full distension can be mimicked using this cell model. The partial distension mode might correspond to the kiss-and-run mechanism of release from secretory cells, which has been proposed to be the major pathway of exocytosis in neurons and neuroendocrine cells.
Bioanalytical Samples - Electrochemical, Fluorescence/Luminescence, Sensors and Lab-on-a-Chip Tech

Development of Micropyramid Array Electrode for Quantitative Detection of Biomolecules

Immunochromatography

Rapid and simple measurement system of biomarkers such as luteinizing hormone and sexual hormones is required in the medical fields especially in production of livestock. Immunochromatography is one of the rapid and simplest immunosensors available, however it is not suitable for quantitative detection of biomarkers. Recently electrochemical detection technique is applied to immunochromatography for providing quantitative capability. The contact between nitrocellulose membrane and electrode is very important to improve the sensitivity. In this regards, we tried to fabricate micropyramid array electrode to improve the contact area between the membrane and electrode, and further evaluated its sensitivity to the ferricyanide ion as an electrochemical mediator by cyclic voltammetry.

Micropyramid array is fabricated with microfabrication process. Firstly, 3D photosensitive resist pattern was formed by grayscale lithography using maskless lithographic equipment. Then the photosensitive resist pattern was transferred to silicon wafer by reactive ion etching. Subsequently, Au thin-film electrode is formed on the micropyramid array by radio-frequency magnetron sputtering. The base length of micropyramid is 20 μm on a side, and its height of 10 μm. We could successfully fabricate the various pyramid array pattern easily, because of employing maskless lithographic equipment, which ultimately cut the cost and time for fabricating photomask. We evaluated the micropyramid electrode array-immunochromatography for measuring the target hormone based on sandwich assay. Target hormone is sandwiched with primary antibody and secondary antibody labeled with glucose oxidaze at the test line on the membrane. After that, glucose and Fe(CN)₆³⁻ are added and Fe(CN)₆⁴⁻ is produced by the enzyme reaction. We measured this Fe(CN)₆⁴⁻ by electrochemical detection. The detail will be discussed. This work was supported by Council for Science, Technology and Innovation (CSTI), Cross-ministerial Strategic Innovation Promotion Program (SIP), “Technologies for creating next-generation agriculture, forestry and fisheries” (funding agency: Bio-oriented Technology Research Advancement Institution, NARO)

Keywords: Biosensors, Electrochemistry, Microelectrode
Application Code: Nanotechnology
Methodology Code: Microfluidics/Lab-on-a-Chip
Pollutants, drugs and other xenobiotics can undergo metabolic reactions mediated by cytochromes P450 and other metabolic enzymes, resulting in reactive metabolites that may cause genetic damage. Metabolite-related genetic damage generally is of two types: (1) adducts of DNA, in which the highly reactive metabolites covalently bind to the nucleobases to form adducts, resulting in unraveling of DNA and exposing 2′-deoxyguanosines (dG); (2) oxidative damage, in which the metabolite-related reactive oxygen species (ROS) reacts with nucleobases to form oxidized nucleobases such as 8-oxo-7-hydro-2′-deoxyguanosine (8-oxodG). Both adducted and oxidized nucleobases may eventually lead to genetic mutations and carcinogenesis.

Here we describe a microfluidic electrochemical array to detect adduction and oxidation in intact DNA simultaneously. The eight-sensor array is incorporated into a 60 L microfluidic channel connected to a pump and sample valve. The array features sensors coated with ruthenium bipyridyl poly(vinylpyridine) chloride [Ru(bpy)2(PVP)10Cl]+ films and osmium bipyridyl poly(vinylpyridine) chloride [Os(bpy)2(PVP)10Cl]+ assembled layer-by-layer with polyions. dG in intact DNA is oxidized by [Ru(bpy)2(PVP)10Cl]+ and 8-oxodG electively oxidized [Os(bpy)2(PVP)10Cl]+, providing separate electrocatalytic electrochemical responses of DNA characteristic of adduct reactions and of DNA oxidation. The same adducted and oxidized products in intact DNA can also be confirmed by high performance liquid chromatography - tandem mass spectrometry (HPLC-MS/MS).

Keywords: Bioanalytical, Electrochemistry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Electrochemical detection of electroactive molecules is a quantitative and sensitive methodology to characterize neurotransmitter release in vitro and in vivo. Enzymatic immobilization onto an electrode surface offers a route to indirectly detect non-electroactive neurotransmitters, such as glutamate, which are not able to be electrochemically detected with unmodified electrodes. However, fast diffusion away from the electrode surface and biological reuptake of analytes can render electrochemical biosensing of neurotransmitters challenging in vitro and in vivo. Here, we describe a bienzymatic system for amplification and electrochemical detection of L-glutamate within gold gapped electrodes. Firstly, glutamate oxidase, which converts L-glutamate into 2-oxoglutarate and hydrogen peroxide, is covalently attached to one of two individually-addressable electrode pairs within a microfabricated gold interdigitated electrode array. A second enzyme, glutamate-pyruvate transaminase, which converts 2-oxoglutarate and L-alanine back into glutamate, is immobilized on the second set of electrodes. Due to the small spacing between each type of electrode (1-2 μm), cycling of enzymatic products will occur and results in an enhancement of electrochemically-detectable hydrogen peroxide one electrode pair under a negative bias. We will show efforts to characterize redox cycling within the gapped electrodes as a function of electrode width and separation and methods to controllably immobilize each enzyme type onto a separate electrode set via polyaniline/glutaraldehyde coupling.

Keywords: Biosensors, Electrochemistry, Electrode Surfaces, Microelectrode
Application Code: Bioanalytical
Methodology Code: Electrochemistry
A Rapid and Sensitive Nano-Immunosensor for Point-of-Care Diagnostic of Bilharzia in Kenya

Bilharzia is one of the Neglected Tropical Diseases (NTDs), a group of chronic disabling infections affecting more than a billion people worldwide, mainly in Africa and mostly the poor. In Kenya, these NTDs affects more than 50% of the population fueling the vicious circle of poverty and stigma that leaves people unable to work, go to school, or participate in family and community life. Highly sensitive detection and accurate analysis is essential for the early detection, treatment, and management of these diseases. Current methods of detection rely on microscopic detection which is tedious, unreliable and suffers poor sensitivity. In this work, a Nano-based immunosensor for early detection which rely on nano-immunological response between an antibody against Bilharzia conjugated to nanoparticles and Bilharzia antigen will be reported. The conjugation of the antibodies with nanoparticles combines the unique properties of the nanoparticles with the specific and selective recognition ability of the antibodies to antigens. The hybrid product has improved cellular uptake as well as the major intracellular stability and may show versatility and specificity with improved analytical signal important for rapid, sensitive and real-time point of care diagnosis.

Keywords: Bioanalytical, Characterization, Electrochemistry, Electrodes
Application Code: Bioanalytical
Methodology Code: Sensors
Fast scan cyclic voltammetry (FSCV) is one of a few analytical techniques which can easily make chemical measurements with submicrometer spatial resolution and subsecond temporal resolution. Because of this, it is useful for making measurements of the response dynamics of dopamine (DA) in living brains. Over the past five years, we have developed the understanding that the DA response kinetics of carbon fiber microelectrodes during in vivo FSCV experiments are strongly influenced by the local biochemistry of the working electrode recording site. We have divided evoked DA responses into two categories – Fast and Slow. Fast DA responses are those which show a DA signal upon 200 ms of 60 Hz, 250 uA stimulus of the medial forebrain bundle. Slow DA responses fail to show a DA signal under these conditions, but do give a response upon continuation of these stimulus conditions in time beyond 200 ms. Here, we have measured the vertical dimension of fast and slow DA domains in the rat striatum, and mapped their anatomical locations. We found the average vertical dimension of fast DA domains to be 1.1 mm, and the average vertical dimension of slow DA domains to be 1.9 mm. We also found that measurements of evoked dopamine made in the dorsolateral striatum were significantly faster than measurements made in the dorsomedial striatum.
Whispering gallery mode (WGM) resonators enable the label-free detection of analytes based on refractive index sensing. We recently demonstrated a large scale multiplexed imaging platform where hundreds of resonators are simultaneously characterized by coupling a fluorescent dye to the resonator surface. This scheme was used to quantify several biomarkers of ovarian cancer with detection limits comparable to ELISA. Recently, we extended this technique by developing an evanescent scattering approach for characterizing the WGM resonances. Moreover, since signal levels scale with excitation power, measurements can be done with high temporal resolution using less expensive imaging equipment. This approach, therefore, offers promise for developing rapid and inexpensive sensing platforms for the multiplexed detection of disease biomarkers. Integration of WGM biosensing with capillary electrophoresis (CE) for analysis of serum proteins will be presented. The integrated platform enables specific detection of biomarkers as they elute from the CE column, which is being developed to aid in the diagnosis multiple myeloma.

Keywords: Biosensors, Capillary Electrophoresis, Lab-on-a-Chip/Microfluidics, Spectroscopy

Application Code: Bioanalytical

Methodology Code: Sensors
Biogenic amines (i.e. arginine, citrulline, aspartic acid, histamine, glutamic acid and taurine) are important compounds that play a role in a number of physiological processes including neurotransmission and inflammation. Determination of these amines in biological matrices is valuable for a better understanding of their functions in physiological and biochemical processes. Presently, capillary electrophoresis with laser induced fluorescence detection (CE-LIF) was employed to determine the trace levels of these amines in biological samples, as this technique can provide high sensitivity and separation efficiencies. Pre-column derivatization with naphthalenedialdehyde (NDA) in the presence of cyanide was employed to produce fluorescent cyanobenzoisoindole (CBI) derivatives. The procedure was accomplished by combining 6.6 µM of each amino acid standard, 20 mM borate (pH 9.0), 5mM NDA and 10 mM NaCN. Due to the high degree of structural similarities between the CBI derivatives, it was necessary to optimize the CE separation by varying buffer additives and the separation voltage. A good separation of the amines was achieved in 17 min in 20 mM borate (pH 9.0) containing 1.4 mM sulfobutylether-β-cyclodextrin (SBE-β-CD) and 10% DMSO using a separation voltage 25 KV and a capillary with an effective length of 62 cm and an inner diameter of 50 µm. Excitation was accomplished using a diode laser (λex = 440 nm and λem = 490 m). A method for the determination of the biogenic amines in microdialysis samples is currently under investigation. The ultimate goal is to transfer the method to microchip format for on-line analysis.
Traumatic brain injury (TBI) is a major cause of mortality and disability in young people around the world.[superscript 1] The initial trauma affecting TBI patients is determined at the time of injury, before medical intervention, but is generally followed by a secondary injury occurring a few days later, while the patient undergoes intense clinical treatment. Ischemia and Spreading Depolarisation waves (SD) are important secondary insults that can be detected while patients are being monitored in the intensive therapy unit (ITU).[superscript 2]

We are developing a miniaturised wireless microfluidic biosensor analysis system that ultimately can be mounted on the patient. On-line microdialysis will be used to sample the cerebrospinal fluid (CSF) and the resulting dialysate will be monitored for changes in levels of key metabolic markers of ischemia and SD, such as glucose, lactate and pyruvate. The system consists of wireless microelectrode-based amperometric biosensors within a microfluidic flow-cell. This novel wearable system will allow monitoring patients to take place earlier, during the initial intensive periods of treatment.

Using a calibration microfluidic board to mimic metabolite changes in the CSF dialysate, preliminary results recorded with a wireless potentiostat will be presented and compared to those obtained with our existing wired biosensor.[superscript 2]

We thank the EPSRC for funding.

[b]References: [/b]

Carbon nanostructures offer the unique advantages of large surface area and conductivity that are suitable for electrocatalytic reactions and biosensors. Our objective is to devise scalable, stable biocatalytic systems representing complex human liver microsomes (HLM) incorporated with nanomaterials. HLM play an important role in the oxidative metabolism of drugs, and hence, studying liver microsomes by protein film voltammetry has gained significant attention at present. Liver microsomes contain membrane-bound major drug metabolizing cytochrome P450 enzymes (CYPs) and their redox partner protein, CYP-reductase (CPR). The in vivo metabolic process involves electron transfer from NADPH to the heme center of cytochrome P450 enzymes (CYPs) mediated by CYP-reductase (CPR). In the present study, we constructed a bioreactor featuring HLM and multi walled carbon nanotubes (MWNT) on the surface of graphite electrodes. The electrochemical and electrocatalytic properties of this novel biocatalytic film will be presented.

Acknowledgements: Financial support by Oklahoma State University is greatly acknowledged.

Keywords: Bioanalytical, Chemically Modified Electrodes, Electrochemistry, Electrode Surfaces
Application Code: Bioanalytical
Methodology Code: Electrochemistry
**Session Title**
Bioanalytical Samples - Electrochemical, Fluorescence/Luminescence, Sensors and Lab-on-a-Chip Tech

**Abstract Title**
Electrochemical Study of 1,10-Phenanthroline-5,6-dione and Glucose Oxidase Modified Graphite Electrode

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**Abstract Text**
Glucose oxidase (GOx) based electrodes are mostly used in the design of glucose biosensors [1] or glucose powered biofuel cells. GOx-based electrodes are suitable for the generation of electrical current, which in some concentration range is proportional to glucose concentration in the sample [ ]. In order to increase the current generated by GOx-based electrodes redox mediators capable to transfer electrons from the active site glucose oxidase to the electrode are needed. To investigate properties of layers that are used for bioanalytical purposes, electrochemical methods, including electrochemical impedance spectroscopy can be applied. The aim of this research was advanced EIS-based evaluation of 1,10-phenanthroline-5,6-dione and glucose oxidase modified electrodes. 1,10-Phenanthroline-5,6-dione and glucose oxidase modified electrodes were analysed at different modification stages by the electrochemical impedance spectroscopy (EIS) method [2]. The gathered impedimetric data was evaluated applying advanced equivalent circuits. The study demonstrated redox mediating properties of 1,10-phenanthroline-5,6-dione deposited on graphite electrodes.

Acknowledgement: The work was supported by Research Council of Lithuania, Support to research of scientists and other researchers (Global Grant), Enzymes functionalized by polymers and biorecognition unit for selective treatment of target cells (NanoZim’s), Project Nr. VP1-3.1-ŠMM-07-K-02-042.


**Keywords:** Biological Samples, Electrochemistry, Electrodes, Enzyme Assays

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
The kinetics of dopamine release and uptake by neurons in the nigrostriatal pathway of anesthetized rats was studied by artificially evoking dopamine release via in vivo electrical stimulation. Dopamine release was recorded using fast scan cyclic voltammetry at carbon fiber microelectrodes. Drugs facilitating dopamine release (raclopride) and inhibiting uptake (nomifensine) were administered, allowing the dopamine overflow resulting from single-pulse stimulations to be resolved. Under these conditions, it was determined that the amount of dopamine released per stimulus pulse decreases with the application of additional pulses. Thus, dopamine release per stimulus pulse is not a constant, as has been assumed by established mathematical models; dopamine release per pulse is in fact dependent on the number of stimulus pulses applied. Differences in the plasticity of kinetic fast and slow sites were observed, with the amplitude of the response per number of pulses decaying much more quickly in slow sites than in fast, even after the application of the D2 antagonist raclopride, suppressing autoinhibition. Additionally, the resolution of one pulse stimulations allowed the size of the gap between the electrode surface and the dopamine-releasing terminal to be estimated. A single pulse was moved in time relative to the data collecting voltage scans, and a gap size of between 3.0 and 3.6 [micro]m was calculated by determining which pulse time differences resulted in a visible dopamine CV at the first voltage scan after the stimulus application.

Acknowledgement:
This project was supported by NIH grant number MH075989.

Keywords: Bioanalytical, Electrochemistry, Neurochemistry, Voltammetry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Our previously developed electrochemical proximity assay (ECPA) is a direct-readout method for analyzing proteins in the femtomolar range. In ECPA, aptamer or antibody-oligonucleotide probes are used to selectively bind to specific proteins. By coupling this binding to other oligonucleotides, one can significantly increase the stability of hybridization of thiolated-DNA (immobilized on gold electrode) and methylene-blue labeled DNA (MB-DNA). This increases the amount of MB-DNA near the gold electrode, which increases the redox current in square-wave voltammetry (SWV) scans. SWV current is thus proportional to the protein concentration. Since the process involves multiple DNA hybridizations, it is temperature sensitive. In this work, we report ECPA responses as a function of temperature using a custom-built temperature control system. To effectively control the temperature, we built a thermal regulator, controlled by an in-house written LabVIEW application. We employed the DNA-loop model which we used in our previous work to verify signal and background currents in the temperature range of 15-50 oC, with a 1 oC increment. We observed that the background was completely denatured by 30 oC, while the signal was stable (DNA hybridization) and increasing (electrochemical increase due to temperature) through a maximum at 25 oC, making S/N ratio very high at this temperature. Beyond 25 oC, the DNA-loop hybridization began to melt, with the transition centered at 30.1 oC. We were even able to observe melting of the DNA anchor from the thiolated surface strand, centered at 48.5 oC. This customized thermal regulation system should enable a greatly improved fundamental understanding of the binding and readout of signal and background in ECPA. In the short term, it should allow significant enhancements in the ECPA signal-to-background ratio.

Keywords: Analysis, Bioanalytical, Biosensors, Electrochemistry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Though advances in breast cancer diagnostics and treatment have led to decreased death rates, there are limitations to current methods. Mammography is an invasive procedure that can miss up to 20% of cancers, and false positives lead to patient anxiety and potential overtreatment. Furthermore, current blood biomarkers such as CA 15-3 and CEA are not suitable for early detection. The goal of this work is to detect biomarkers indicative of early stage breast cancer in serum. Stage-indicative breast cancer proteins may be present in serum at low concentrations and undetectable by current methods such as a traditional plate ELISA. This sensitivity barrier can be overcome by utilizing single molecule arrays (SiMoAs) as a detection platform, which uses digital counting to measure single protein molecules; this method can measure concentrations of proteins 100-1,000X lower than traditional ELISA detection limits. SiMoA technology can be applied to detecting low abundance proteins toward a noninvasive, ultrasensitive blood test for breast cancer. The work presented includes assay development of several potential breast cancer biomarkers, as well as tests in healthy and patient serum and urine.

This work was funded by DOD BC100510 (W81XWH-11-1-0814).
Application of Zirconia Phases for Structurally Similar Compounds and Other Difficult HPLC Separations

Method development can be challenging for structurally similar compounds and complex ionic mixtures. Additionally, validation methods often require a column of orthogonal selectivity be used to ensure no impurities are missed. Zirconia based phases, with their inherent surface chemistry differences, offer a dramatically different selectivity when compared to silica and polymer phases. Here we investigate two of the most unique zirconia-based phases, a carbon-clad zirconia phase and a crosslinked polyethyleneimine coated zirconia phase.

To demonstrate the differences between the zirconia based, carbon-clad ZirChrom[registered]-CARB phase, we highlight a selectivity comparison versus a typical silica bonded phase for 22 non-electrolyte solutes with varying chemical properties. The analysis of a set of six structurally similar sulfated steroids will be explored to demonstrate how this orthogonal selectivity can be successfully applied.

The unique multi-modal surface chemistry of the zirconia-based, polyethyleneimine coated, ZirChrom[registered]-SAX phase can be helpful in the analysis of complex ionic samples. A discussion of the surface chemistry and multi-modal separation mechanisms of the phase will be presented. Finally the method development for a separation of six water soluble vitamins will be included as an example of how the multi-modal selectivity of ZirChrom[registered]-SAX can resolve a complex ionic mixture.

Keywords: HPLC, Pharmaceutical, UV-VIS Absorbance/Luminescence
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
### Abstract Text

This paper described a method for monitoring 3-Methyl-5-Isopropylhydantoin (MVH), a biomarker of the degradation of dimethylformamide (DMF) in hemoglobin. Cleanert MAS-B Plate with 96-well format was used for rapid clean-up of the samples. 3-Methy-5-isobutylhydantion (MIH) was applied as internal standard to quantify MVH in hemoglobin. A clean fraction was obtained for high throughput monitoring of MVH and detected by LC-MS/MS without further concentration. Recoveries for MVH were range from 100.4% to 102.5% with RSDs were <4.6%. LOD of MVH and MIH were 1.0ng/mL. The data highlights Cleanert MAS-B 96-well Plate with 25mg sorbent in each well is suitable to remove the matrix of hemoglobin which might interfere in the analysis of MVH by LC-MS/MS. The usage of the 96-well plate is sufficient for high throughput sample clean-up procedure prior to LC-MS/MS. This study provided a solution for monitoring cumulative exposure to DMF.

### Keywords:
- Biopharmaceutical
- Chemical
- Liquid Chromatography/Mass Spectroscopy
- Plasma

### Application Code:
- Pharmaceutical

### Methodology Code:
- Liquid Chromatography/Mass Spectrometry
Abstract Text

Established a rapid clean-up procedure for detecting Indomethacin and Ibuprofen from human plasma. The method employed Cleanert PEP MicroPlate for sample purification which was a water-wettable polymer-based SPE sorbent. The extraction procedure using a modular design μElution plate allowing the number of wells to match samples numbers being processed. Analyte concentrations ranged from 5 to 50ng/mL, the analytes were eluted by 100μL acetonitrile and detected with LC-MS/MS. This ensured little or no analyte dilution compared with the original sample volume. In addition, the elution volume ensured evaporation and reconstitution time was minimised and throughput improved. Recoveries for the two analytes were >85% for Indomethacin and >90% for Ibuprofen. LOD of Indomethacin and Ibuprofen were 0.5ng/mL and 1.0ng/mL respectively. The data highlights the minimum elution volumes that can be achieved from the 5mg sorbent mass and the versatile nature of the modular plate design.

Keywords: Biological Samples, Chemical, Liquid Chromatography/Mass Spectroscopy, Plasma
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Established a method for detecting Telmisartan from human plasma. The method employed Cleanert SLE Plate for sample purification. The technique mimics the characteristics of liquid-liquid extraction (LLE), but unlike LLE lends itself to high throughput and automation. Cleanert SLE packed with a diatomaceous earth, each well provides a support to absorb the aqueous sample, whilst leaving the analytes on the surface - there are no interactions between the analyte and the diatomaceous earth. Analytes were subsequently eluted with a water immiscible organic solvent. The plate was packed with 600mg of sorbent, allowed a maximum load volume to 600 l. The top frit of the plate has been surface modified to a more hydrophilic nature which contributed to plasma samples loading. Recoveries for Telmisartan was 83.6 ~98.9%. The RSD was <4.5%. Linearity checks across the full concentration range were good. LOD of Telmisartan was 0.1ng/mL.

Keywords: Biological Samples, Chemical, Liquid Chromatography/Mass Spectroscopy, Plasma
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
As the medical cannabis industry evolves, applications for chemical analysis of cannabis concentrates are growing. In addition to being simply vaporized by the end user, concentrates may be used to prepare alternate dosage forms such as tinctures, topical salves, and transdermal patches. Like all pharmaceutical products and regardless of the final dosage form, potentially harmful residual solvents from the manufacturing process must be minimized and monitored.

While sample dissolution methods for residual solvents analyses exist (e.g. USP <467>), for some cannabis concentrates, it is extremely difficult to achieve dissolution in common, late-eluting solvents that will not interfere with headspace analysis. Additionally, complex matrices such as cannabis concentrates usually require matrix-matched standards to ensure accurate quantification. Through the use of full evaporation technique-headspace gas chromatography (FET-HS-GC), accurate quantification for residual solvents can be achieved to low ppm levels in concentrates without the need for matrix-matched standards.

A method was developed to quantify levels of isobutane, butane, methanol, pentane, ethanol, acetone, isopropanol, hexane, chloroform, benzene, heptane, and toluene using FET-HS-GC. Adequate resolution was achieved for all compounds with an analysis time of less than 10 minutes. Calibration curves were prepared from 5 – 500ppm residual solvents, assuming 20mg of sample. Linearity was acceptable for all compounds, with r² values ranging from 0.992 for isobutane to 1.000 for heptane. Signal-to-noise ratios at 10ppm ranged from 5.3 for isobutane to 170 for toluene.

This presentation will introduce the full evaporation technique, discuss method optimization parameters for headspace analyses, and present quantitative data collected for the work outlined above.

**Keywords:** Drugs, Gas Chromatography, Headspace, Method Development

**Application Code:** Pharmaceutical

**Methodology Code:** Gas Chromatography
Residual host cell proteins are an immunogenicity concern for biotherapeutics. Recently the USP released an updated chapter for residual host cell protein measurement in biopharmaceuticals, including guidance for testing the residual amount of these proteins in biotherapeutic products. It is a regulatory requirement to demonstrate clearance of residual host cell proteins following standard processing of biological therapeutics. Greater than 1000 proteins are routinely detected in the cell culture supernatant, and assessing coverage of the polyclonal reagent for a complete match is challenging. The industry gold standard approach is to utilize 2D-silver and western blotting to assess the coverage of polyclonal antibody reagent used in standard ELISA test method. 2D silver/western blot method is time consuming, tedious, and prone to reproducibility error. Analysis can vary widely resulting in significantly different coverage assessment values which in turn can cause concern to the applicability of the ELISA method. To make the 2D-silver/western more reliable, a systematic approach has been developed to assess the analytical variability and more accurately report the results. Additional techniques to assessing the polyclonal reagent and its coverage to process host cell proteins are also being developed including 2D-DIGE and 2D LC-MS. These emerging techniques have been investigated to provide a more thorough assessment of the host cell protein polyclonal reagent coverage and to improve the reproducibility of the host cell protein reagent.

Keywords: Biopharmaceutical, Biotechnology, Characterization, Protein
Application Code: Pharmaceutical
Methodology Code: Other
Preparative scale liquid chromatography can be challenging to the isolation chemist in many ways, but effective sample loading directly influences the success of compound isolation. While the objectives of prep chromatography include achieving high mass load on the column and employing rugged generic chromatography, two conflicting principles almost always play a role in the approach used to realize these objectives. First, strong solvents dissolve samples but distort chromatographic peaks due to the inability of the sample to properly interact with the column packing. Second, weak solvents as sample diluent give good chromatography but do not dissolve samples at high concentration. Low sample concentration ultimately results in large injection volumes which are difficult to handle and generally result in poor chromatography. Although these challenges are well known among chromatographers, with easy modifications to the HPLC system plumbing these issues can be addressed satisfactorily. In this study, we illustrate and compare different techniques for improving the efficiency of loading large volumes of sample for compound isolation. The impact of each technique on the overall purification efficiency will be discussed.
As USP global initiative to modernize existing monographs across all compendia, objectives and criteria for the monograph modernization are discussed. Identification, assay and organic impurities tests in the USP monograph are highlighted with modern analytical techniques for the modernized monograph. A fast and reproducible Hydrophilic Interaction Chromatography (HILIC) method coupled with Charged Aerosol Detector (CAD) is developed for non-chromophoric hydroxylamine in metoprolol succinate and related solid dosage drug formulations. This method allows rapid quantitation of non-chromophoric metoprolol impurities in the presence of metoprolol succinate (a selective receptor blocker used in treatment of several diseases of the cardiovascular system, especially hypertension) and its potential degradants. In the presentation, USP internal monograph development strategy and monograph review process are also addressed.
Dichloromethane (DCM) is both the lifeblood and main chemical waste of many pharmaceutical medicinal chemistry laboratories employing flash chromatography. The solvating power of DCM for many organic compounds when combined with its low boiling point make it an ideal non-polar solvent for this application. However, DCM is not without its health risks to the scientist or specialized solvent waste concerns. This paper will discuss a uniquely novel approach utilizing carbon dioxide to completely eliminate and/or at the very least, greatly reduce the amount of DCM and other non-polar solvents currently employed flash chromatography purifications worldwide.

Keywords: Chromatography, Instrumentation, Isolation/Purification, Prep Chromatography
Application Code: Pharmaceutical
Methodology Code: Separation Sciences
Chiral amines are powerful pharmacophores for creating new pharmaceuticals due to their inherent amount of structural information and their ability for hydrogen bonding, yet their analysis remains a challenge. Recently a novel class of chiral stationary phases (CSP) based on cyclofructan has been developed. Cyclofructans are cyclic oligosaccharides that possess a crown ether core and pendent fructofuranose moieties, which have been successfully derivatized. Aliphatic derivatives of this sort have shown “class” selectivity towards primary amine containing enantiomers. This is well documented. However, lab technicians often prefer to only use the columns “on-hand” or those that are already in their chiral screening unit. Herein, we show a simple comparison of the increased number of hits obtained by using one cyclofructan based column for screening 30 chiral primary amine separations versus using 6 cellulose and amylose based columns. Additionally, the time saved from only screening 1 column instead of 6 columns is demonstrated. Other topics that will be discussed include the amount of solvent that can be saved using the proper screening tools, any complementary separations that are observed between phases, and the effect of using manufacturer recommended screening mobile phases.

Keywords: Chiral, Chiral Separations, HPLC, Liquid Chromatography
Application Code: Drug Discovery
Methodology Code: Liquid Chromatography
This poster was a comparative study about 3 kinds of sample pretreatment processes to extract Arachidonic Acid (AA) from plasma, which involved Protein precipitation 96-well plate, Brand W 96-well plate and Cleanert MAS-M 96-well plate. Protein precipitation method enjoyed a convenience due to its minimum procedures, but its recoveries of AA were 129.32%~149.02%, implying the worst purification effect which caused the matrix enhancement on mass spectrum. The recoveries of AA on Brand W 96-well plate were 5.45%~70.15%, while the recoveries of Cleanert MAS-M 96-well plate were 99.19%~106.38 which ensured an extraction procedure without reconstitution to support a rapid, high throughput assay of AA in plasma.

Keywords: Drug Discovery, Liquid Chromatography/Mass Spectroscopy, Plasma, Sample Preparation

Application Code: Drug Discovery

Methodology Code: Liquid Chromatography/Mass Spectrometry
USP methods are used widely in the pharmaceutical industry and are often not optimized for use with modern instrumentation and packing materials. As it becomes increasingly important to boost throughput in the modern laboratory, new instrumentation and smaller particle size packing materials are allowing scientists to achieve these goals while also realizing cost savings in terms of man-hours and solvent usage. In this poster we investigate the use of 3um YMC-Triart C18 and 2.7um YMC-Meteoric Core C18 stationary phases for improving a USP assay method for chlorhexidine gluconate.

Abstract Text

Keywords: HPLC, Liquid Chromatography, Optimization, Pharmaceutical

Application Code: Pharmaceutical

Methodology Code: Liquid Chromatography
## Abstract Title

**Extraction of $\beta$-blockers from Small Volume Biological Fluid Samples Using a New Versatile SPE 96-well Plate Format**

## Abstract Text

With the growth of high blood pressure and heart disease population, related drugs such as $\beta$-blockers become the focus of attention. So a simple and efficient sample preparation technique is necessary. This study was carried out by using solid phase extraction cartridges with a format of 96-well plate and LC-MS/MS detection for the analysis of metoprolol and propranolol in human plasma. The plasma was spiked with the two $\beta$-blockers at a concentration range from 1 to 5 ng/mL. The LODs of metoprolol and propranolol was 0.02 ng/mL and 0.1 ng/mL. The method is rapid and efficient with the recovery range from 84.2% to 92.2%. The results indicate that with 5 mg packing material of Cleanert PEP with a format of 96-well plate $\beta$-blockers in human plasma can be extracted effectively and eluted with a small volume of the eluant.

## Keywords
- Drug Discovery
- Liquid Chromatography/Mass Spectroscopy, Plasma, Sample Preparation

## Application Code
- Drug Discovery

## Methodology Code
- Liquid Chromatography/Mass Spectrometry
Evaluation of a Low-Cost Mass Spectrometer

A low-cost, compact single-quadrupole mass spectrometer was evaluated for pharmaceutical analysis in supporting method development of stability indicating assays and cleaning verification testing. The advantages found were: easy to use and maintain, sensitive, and excellent software integration of UV and MS data in Acquity UHPLC Empower systems with automated annotation and reporting functions. Some of limitations found were: not very “portable”, electrospray ionization only, no adjustment needed or possible, and “not yet integrated with other vendors’ HPLCs”. The poster will include a description of the system, schematics and an Empower instrumental method. Case studies shown will include 1. a stability-indicating assay with a peak table containing UV and MS data plus system suitability values for each peak; 2. A generic cleaning verification method of 12 new chemical entities with automated annotations of UV and MS spectra indexed to each peak and performance data of the UV and MS channels.

Keywords: Data Analysis, Liquid Chromatography/Mass Spectroscopy, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Mass Spectrometry
Pharmaceutical - LC, LC/MS, GC

Comparative Determination of “Tar”, Nicotine, and Carbon Monoxide Smoke Deliveries of Little Cigars and Traditional Cigarettes

Little cigars (LC) are one of several tobacco products not currently regulated under the authority given by the Family Smoking Prevention and Tobacco Control Act signed in 2009. With the exception of being wrapped in paper derived from tobacco, they physically resemble cigarettes (e.g., size and shape). There is little information available on LC so a study was conducted to compare similarities and differences within the tobacco filler and resulting smoke chemistries. Smoke collected from 15 brands of LC—using the ISO regime (35 mL puff volume; 2 sec puff duration; 60 sec puff interval) and Canadian Intense (CI) regime (55 mL puff volume; 2 sec puff duration; 30 sec puff interval)—was analyzed for “tar”, nicotine, and carbon monoxide (TNCO) deliveries using gravimetric, gas chromatography-flame ionization detection (GC-FID), GC-thermal conductivity detection (GC-TCD), and non-dispersive infrared (NDIR) analyses, respectively. Although a CORESTA recommended method exists for TNCO measurements in cigar smoke, the ISO and CI smoking regimes were used in order to compare LC smoke data to traditional TNCO smoke data from some of the top, select U.S. cigarette brands. Among the brands tested nicotine levels between LC and cigarettes were comparable under ISO and CI smoking regimens; however, tar deliveries for LC were generally higher (ISO: LC 18.5±5 mg; CI: LC 46.5±4.8 mg; ISO: cigarettes 12.2±4.7 mg; CI: cigarettes 32±4.6 mg tar).

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Keywords: Gas Chromatography, Natural Products

Application Code: Other

Methodology Code: Gas Chromatography
The Cannabis plant has been known for centuries for the medicinal properties of its extracts and the medical benefits. Using Centrifugal Partitioning Chromatography (FCPC by Kromaton), an interesting approach has been developed to obtain pure fractions of Cannabinoids. The process was optimized for methanol extracts containing a mixture of Tetrahydrocannabinol (THC), Cannabidiol (CBD), and Cannabigerol (CBG) and respective isomers. Due to the selective partitioning nature and discrete stagewise operation of the FCPC device, pure fractions of the constituents with very similar molecular structures can be obtained in semi-preparative, preparative, and production quantities.
Improvements in the Characterization of Monoclonal Antibody Primary Structure Using Wide Pore Superficially Porous Particles

Abstract Text

With the continued importance of biotherapeutic proteins, comprehensive characterization is a prerequisite. It is of paramount importance for the production process to be highly consistent and robust, and that any change in process related impurities that could impact clinical outcomes and immunogenicity are avoided. Characterization of monoclonal antibody (mAb) primary structure using reversed-phase liquid chromatography is a key activity in bio-pharma discovery, development and QA/QC. Comprehensive characterization requires the analysis of intact, heavy and light chains, and Fc and Fab regions of the antibody using liquid chromatography often coupled with high resolution mass spectrometry. Increases in the speed and resolution offered by the HPLC columns used in these separations facilitate improvements in the quality of characterization data.

The speed and resolution of HPLC analyses can be improved through the use of HPLC columns packed with superficially porous particles (SPP). Compared to similarly sized totally porous particles, SPP columns offer improved efficiency and performance. This is primarily due to a shorter mass transfer distance and substantially narrower particle size distribution. The majority of existing SPP columns range in pore size from 90Å to 120Å. More recently, however, wide pore SPP columns have been made available with pore sizes >300Å. These wide pore SPP columns are ideally suited to analyze large biomolecules, such as mAbs.

This report will show the advantages that optimized particle morphology and bonding chemistry used in reversed-phase HPLC columns can bring to challenging mAb separations.

Keywords: Bioanalytical, Biopharmaceutical, Liquid Chromatography, Liquid Chromatography/Mass Spectroscopy

Application Code: Bioanalytical

Methodology Code: Liquid Chromatography
Pharmaceutical - LC, LC/MS, GC

Fast Separation of Glycans Using HILIC Chromatography

Glycosylation is a dynamic biologically active post-translational modification which is a critically important and elaborately complex post-translational modification that requires control, monitoring and understanding during all phases of glycoprotein drug development, processing and manufacture. The structural profile of both N linked glycan must be elucidated completely during the development of new biological pharmaceutical products and throughout the development of Biosimilars or Biological follow-on products.

Utilizing novel glycan sample preparation methods and detection by fluorescence and mass spectrometry, we present a total workflow solution for the analysis of human IgG N-linked glycans. Specifically, this work is based on novel HILIC (amide-based) columns which enable high resolution and significantly reduced glycan elution times compared to currently available HPLC technologies. HILIC separations using both sub 2µm fully porous and 2.7µm superficially porous columns were used to demonstrate UHPLC and HPLC high-throughput solutions for the rapid analysis of glycosylated proteins.

Keywords: Biological Samples, Biopharmaceutical, HPLC Columns, Sample Preparation
Application Code: High-Throughput Chemical Analysis
Methodology Code: Liquid Chromatography
When running impurity profile analysis, dynamic range of the detector is one of the most important parameter. Current norms in the pharmaceutical industry require at least 3 orders of magnitude in order being able to quantify 0.1% impurities. Running low dynamic range detector analysis often requires a multi injection sequence with external calibration, since it is impossible to get a single injection chromatogram with a non-saturated major peak, and minor peaks simultaneously. ELSD is a nearly universal technique that should be considered as an advantageous alternative for UV detection in impurity profiling, since response factor is generally tighter than with UV detectors, providing a more accurate picture of impurity profile. However, in some cases, ELSD does not provide enough dynamic range to assess impurity profile within one chromatogram. A new generation ELSD equipped with a patented feature (SAGA™, automated adjustment of the dynamic range) is shown to provide an efficient way to override this limitation, providing more than 4 orders of magnitude of dynamic range in a single setting, allowing the impurity profiling within one injection. This new ELSD feature can also be used advantageously in purification techniques, where higher dynamic range allows better monitoring of the purification process, avoiding saturation of the detector without compromising sensitivity of minor impurities.

Keywords: Detector, Isolation/Purification, Liquid Chromatography, Validation
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Sutherlandia frutescens commonly known as cancer bush is widely distributed throughout southern Africa and is used as a traditional medicine for the treatment of a wide variety of ailments. S. frutescens produces a range of well-known secondary metabolites with biological activity. Although several compounds with medicinal properties have been isolated from extracts of the plant, no reports exist concerning the effect of contaminated soils on the production of such metabolites. In this study S. frutescens specimens were collected from acid rock mine dumps and the possible alteration in the profile of secondary metabolites due to oxidative stress was evaluated by determination of total phenolic compounds, quantification of flavonoids using HPLC, metabolomic profiling using LC-MS and bioactivity of S. frutescens extracts. The antioxidant activity of extracts obtained from plants growing in acid rock mine dumps was significantly higher than the activity of extracts from control plants. This could be attributed to the high levels of total soluble phenolic compounds, kaempferol and quercetin produced by specimens collected from the mine sites. Kaempferol and quercetin are flavonoids with well-known antioxidant activity. Four flavanol glycosides (Sutherlandin A, B, C and D) and the triterpenoid Sutherlandioside B (SU1) were identified using UV spectra and confirmed using TOF-MS. Sutherlandins and Sutherlandiosides are biomarkers of S. frutescens used for quality control. The theoretical masses of Sutherlandins A-D (740.1800, 740.1800, 724.1851 and 724.1851) corresponds to the ions detected using ESI: m/z 739.1720 [M-H]-; m/z 739.1729 [M-H]-; m/z 723.1793 [M-H]- and m/z 723.1783 [M-H]-. The fragmentation pattern is similar to that reported previously for natural populations of S. frutescens. The SU1 is reported to have anticancer activity and the theoretical mass of SU1 (652.4186) reported in literature corresponds to the mass obtained in this study (651.9348).
Retention time precision is extremely important in being able to accurately compare results. Traditionally, retention time precision was always attributed to flow characteristics, such as fluctuation in flow rate and gradient imprecision, in an LC system. However, with UHPLC a point has now been reached where other factors, such as pressure and speed, play major roles. Internal friction results in viscous heating and this can cause non-uniform temperature profiles. At injection the sample is pressurized from one bar to pressures above 1000 bar with the switch of a valve and peak widths can be so narrow that the data collection rate can become insufficient accurately profile the peak. Finally, there are issues with gradient formation at pressures above a 1000 bar. In this work, we will look at all the aspects of a UHPLC system and discuss how chosen parameters can influence retention time. We will show that more factors are important than just pump flow and gradient stability, and that it is possible to generate very accurate retention time precision at the top of the UHPLC operating range.

Keywords: HPLC, HPLC Detection, Liquid Chromatography
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Abstract Text

Accurate and reproducible analytical methods are a critical component of successful drug product development. In parallel to formulation and process optimization, analytical method optimization is conducted to ensure the key critical quality attributes (CQAs) are characterized appropriately. It is critical that optimized methods are in place by the time of process scale-up and Formal Stability Studies (FSS) in order to be sure product decisions are based on high quality data. Quality by Design (QbD) and DOE (Design of Experiment) approaches can be applied during method development to facilitate an understanding of both effects and variation within multiple experimental factors as well as to characterize the overall precision and robustness of the methods.

In this example, the suitability of the assay/degradate method has been evaluated for an active pharmaceutical ingredient (API 1) currently in formulation development as a fixed dose combination (FDC). An HPLC method was initially developed using a one-factor-at-a-time (OFAT) approach, where each variable was changed sequentially until suitable methods were identified. To increase the understanding of method capabilities and robustness, a QbD approach using statistical DOE was employed. Robustness testing was conducted to identify each method’s “design space,” or experimental region in which changes to method parameters will not significantly affect the quality and results of the method. This was done by introducing variations in method parameters and evaluating the impact on method output. Overall method variability, reported as Gauge R&R, was determined by evaluating the sensitivity of key method attributes to variables such as HPLC system, column, standard preparation, and analyst in order to quantify the final method capability.

Keywords: Chromatography, HPLC, Method Development, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Separation Sciences
Abstract Text
Increased efficiency in HPLC columns with fully porous particles (FPPs) can be achieved by using smaller particle size at the cost of an increased back pressure. In comparison, superficially porous particle (SPP) contains a thin porous outer shell with a solid core and provides extraordinary efficiencies. A 2.7-μm SPP provides efficiency of a sub 2-μm FPP but retains the backpressure of a 3-μm FPP. SPPs also has a very small particle size distribution which results in higher packing density and a more uniform bed, providing overall increased efficiency. In this work, chiral stationary phases (CSPs) based on derivatized cyclic oligosaccharides and macrocyclic glycopeptides are bonded to SPPs to provide unmatched efficiency. The chromatographic performance of a CSP bonded to a 2.7-μm SPPs is compared to columns packed with 5-μm and 3-μm FPPs. The difference in selectivity and resolution of analytes at constant elution strength and constant retention are compared for these columns. Greatly reduced analysis time and significant saving in mobile phase when using SPPs are discussed.

Keywords: Chiral Separations, Chromatography, Liquid Chromatography, Modified Silica
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Pharmaceutical - LC, LC/MS, GC

Ultrafast Method Screening for Separation of Enantiomers in HPLC and SFC Utilizing Novel Polysaccharides Type Chiral Stationary Phases Based on Small Particles

Mechanism of chiral separation on liquid chromatography is very complicated, and the separation is made by complex combination of various interactions, such as hydrophobic, hydrogen bonding, dipole-dipole, and [pi]-[pi]. This makes method development of chiral separation difficult. Therefore, the column screening is commonly recognized as the first stage of separation method development. The fast column screening is the key driver for the rapid establishment of separation method.

Recently, we developed chiral stationary phases consisting of polysaccharides derivatives immobilized on 3 [micro]m silica gel particle. The new material is ideal for the fast method screening due to the high column efficiency across a wide range of flow rate. Moreover, it shows separation selectivity identical to conventional materials in 5, 10, and 20 [micro]m particle sizes. This feature enables predictable method transfer from ultrafast separation method to conventional method using 5 [micro]m material, and even to preparative method.

In this poster, we will present an ultrafast method screening of chiral separation utilizing the 3 [micro]m chiral separation column through some applications. We will also show the possibility of further reduction of method screening period by a combination of the column and supercritical fluid chromatography (SFC).

Keywords: Chiral Separations, High Throughput Chemical Analysis, HPLC Columns, SFC
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Highly Efficient Purification of Enantiomers Using Polysaccharide Type Chiral Stationary Phases and Continuous Purification Technology

The role of chiral separation is becoming more and more important especially in pharmaceutical industry, and the demand for isolating an enantiomer with high purity is increasing. However, there are two hurdles to be overcome; difficulty in method development and cost effectiveness of purification.

We have recently developed various chiral stationary phases coated/immobilized with polysaccharide derivatives. The coated phases give great resolution and the immobilized phases offer wide range of solvent compatibility. These phases greatly contribute to reduction of method screening period by combining column screening and mobile phase screening.

The separation method developed at analytical scale can be easily and linearly scaled up to purification from milligrams to kilograms process by using preparative scale column and LC-Forte/R preparation LC system. In addition, the efficacy of purification is improved by applying recycling preparative method of LC-Forte/R. This recycling method is also applicable to cases where ideal resolution is not achieved at method screening stage.

In this poster, we will show an example of method development including column and solvent screening at analytical scale, and then method transferring to purification scale. We will also estimate the purification efficacy of recycling LC method.

Keywords: Chiral Separations, HPLC Columns, Pharmaceutical, Prep Chromatography
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
More than 90% of reversed-phase UHPLC/HPLC analyses have been considered to be feasible using C18 phases. Recently, use of inorganic/organic hybrid particle based C18 phases is increasing due to their high efficiency and good chemical stability/long lifetime, and, so far, a number of hybrid particle based C18 phases have been marketed by various vendors. However, most of those phases have been developed based on a strategy of making a “Standard C18” that has moderate hydrophobicity and hydrophilicity. This sometimes impeded the flexible method development.

In order to enable a wide variety of method development using C18 phase, we developed a new high coverage and fully-endcapped C18 phase based on robust hybrid particle. This phase offers complementing selectivity to standard C18 phases, rendering it efficacious in separating hydrophobic drug substance from structurally similar impurities. Also, its chemical durability, especially under neutral/alkaline conditions, enables the rapid optimization of mobile phase conditions without limitation of the usable pH range.

In this poster, we introduce differential characteristics between this new C18 phase and our standard hybrid based C18, Triart C18. We also show some examples of the efficient method development utilizing these high-coverage and standard C18 phases.

Keywords: High Throughput Chemical Analysis, HPLC Columns, Liquid Chromatography, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
The United States Pharmacopeia (USP) defines allowed adjustments of HPLC and GC parameters in the general chapter <621> chromatography. If the system suitability is met, method parameters can be changed within the allowed limits without revalidation. The general chapter 621 was revised in the first supplement to USP37-NF32 published on February 1st 2014 and became official August 1st 2014. The feature of the new general chapter 621 is that a column packed with small particles can be used as long as column length and particle ratio (L/dp) are kept constant between the designated and modified column. This enables the high speed analysis of USP methods more than ever. In this poster, an adjustment of a USP method for speed will be shown. The USP designates the use of a 4.6 × 150 mm, 5 µm, L1 (ODS) column and a 0.8 mL/min flow rate for the impurity analysis of sulfacetamide. If a 2.0 × 50 mm, 1.6 µm, L1 column is used, L/dp is kept constant, so there is no need to revalidate the method. The new flow rate after method transfer is calculated to 0.47 mL/min according to an equation in the general chapter 621. This high speed method was run on a Nexera X2 UHPLC system. As a result, analysis time and solvent consumption were reduced to 1/10 and 1/15, respectively, with the system suitability requirements met. Additionally, running a USP method on the Nexera X2 UHPLC system will be shown. The assay for timolol maleate ophthalmic solution was run on the Nexera X2 system without changing method parameters. The system suitability requirements were easily met on the UHPLC system similar to a standard HPLC system. This result means that this system is also suitable for those who are running traditional USP methods on a standard HPLC system and are considering the adoption of a UHPLC system for high speed analysis of USP methods in the future.
Abstract Text

The oligosaccharide component of glycoproteins is a key determinant of their function. Changes in the number, type, composition or linkage pattern of these glycans may serve as a biomarker of disease or influence the efficacy of a biotherapeutic product. For this reason, the ability to correctly identify and measure these glycans is of scientific interest, and to do so reliably, quickly and inexpensively is of practical benefit. This work explores direct detection of native glycans as an alternative to the common techniques for glycan analysis that rely on derivatization reactions to render glycans detectable. The lack of a detectable chromophore in native glycans is overcome by using HPLC with charged aerosol detection, a detector that can quantitatively measure any non-volatile compound.

N-linked glycans were released from proteins by PNGase-F. The native glycans were separated by ultra high performance liquid chromatography (UHPLC) on a column that employs both weak anion exchange and reversed-phase separation mechanisms, thus resolving glycans based on charge, isomerism and size. This unique selectivity resolves difficult to separate isoforms that are not resolved by conventional column types. The native glycans were detected directly without derivatization by using charged aerosol detection. Glycans released from various proteins were analyzed including those from bovine fetuin and monoclonal antibodies. Quantitative performance including precision, detection limits and dynamic range is presented. Typical figures of merit include sensitivity at the low-nanogram on-column level, dynamic range over two orders of magnitude, and peak area precision typically less than two percent RSD. By responding directly to any non-volatile compound, charged aerosol detection is able to measure directly native unlabeled glycans, yielding simple, accurate and precise estimates of relative concentration even in the absence of pure primary standards.

Keywords: Biopharmaceutical, Carbohydrates, HPLC Columns, HPLC Detection

Application Code: Pharmaceutical

Methodology Code: Liquid Chromatography
## Abstract

Spectral imaging offers many advantages over more invasive, destructive analytical techniques and yields maps of chemical composition as well as differences in the physical characteristics of the sample, depending on the spectral modality employed. To date, narrow-band methods like Brillouin inelastic scattering spectroscopy have not been adapted for turn-key line-scan or wide-field imaging applications due, in part, to the lack of suitable wavelength filters. The Brillouin modality results from the interaction of incident photons with the acoustic phonons of the sample and Brillouin imaging would be useful for optically mapping the elastic properties of the sample nondestructively. The challenge is developing suitably narrow-band image filters. Recently the Brillouin spectrum has been resolved using a virtually imaged phased array (VIPA), which can be thought of as a modified side entrance Fabry-Perot etalon employing a range of incident angles. In the work presented here, we have developed and characterized several narrow-band VIPAs. A fully generalized dispersion equation has been developed which relates the input optical field to the VIPA response for both isotropic and anisotropic dielectrics. Hence, the dispersion relation is well suited for designing tunable VIPA elements that would enable high spectral resolution in a fixed image plane. In addition to the full theoretical description, validation studies as well as the design of a Brillouin imaging system are presented.

### Keywords

Instrumentation, Light Scattering, Molecular Spectroscopy, Spectrometer

### Application Code

Materials Science

### Methodology Code

Vibrational Spectroscopy
Surface enhanced Raman spectroscopy (SERS) can greatly enhance the signal from the Raman-active molecules that have been adsorbed onto specially prepared metal surfaces. The selectivity and the high sensitivity of SERS make it a superior method to Raman spectroscopy. The advantage of SERS technique is that it greatly enhances the signal from the Raman-active molecules, which have been adsorbed onto specially prepared metal surfaces. Normally the factor by which the signal is enhanced is $10^4$-$10^6$. In certain cases it may be as high as $10^{14}$. Raman Spectroscopy is surface selective and its sensitivity is very low. Nanostructures based on gold and silver are widely used for analytic adsorption in SERS method. In this study, we will discuss the comparative SERS analysis of meta-anilines, ortho-anilines, and para-anilines using silver colloids. The roles of aniline substituents including halogens, nitro, methoxy, methyl, isopropyl, and tert-butyl groups are elucidated by SERS analysis with an excitation wavelength of 785 nm. The SERS enhancement factors and detection limits for aniline and its mono- and di-substituted aniline derivatives are determined and compared. Both the steric requirements of chemisorption and the inductive effects of electron-withdrawal due to the substituent groups affect the signal intensities. The colloidal nanoparticles were also deposited onto copper surface for surface-enhanced infrared analysis (SEIRA) of the aniline derivatives. The characteristics of their adsorption onto metal surfaces and enhancement factors of SERS and SEIRA are discussed in terms of the computational modeling of the aniline structures. The potential for using SERS and SEIRA for environmental monitoring of the aniline derivatives will be presented.

**Keywords:** Analysis, Raman, Spectroscopy, Surface Enhanced Raman

**Application Code:** Process Analytical Chemistry

**Methodology Code:** Vibrational Spectroscopy
Cavity Ring Down Spectroscopy (CW-CRDS) is a technique in which the rate of absorption is measured. Because of its many advantages, it has given possible potentials as a reference instrument with calibration-free. In order to use reference standard instrument, interference from other gas components should be precisely tested. Many commercial instruments have been widely used in the determination of trace gas components in recent years. Nevertheless, their internal correcting procedures of interference have been remained as company confidential. So, it is difficult to study commercial instruments for reference standard instrument.

In order to study and apply CRDS to reference standard instrument, we have constructed a CRDS system of methane with precisely controlled temperature and pressure module. In this presentation, we explained the constructed system and preliminary results. Experimental setup of constructed CW-CRDS are shown in attached Fig. For the determination methane, DFB laser diode (NTT electronics Inc., wavelength, 1650 nm, 15 mW) was used. Optical switch (Agiltron, model, 300 ns nano-speed) with circulator (Flyin Optronics Co.) were also used to switch off the amplified cavity signal and prevent reflected light. And mirrors (CRD Optics, 99.95%) in gas sampling cavity of 30 cm were used and PZT (PI, length, 13 mm) was attached to the exit mirror. InGaAs photo-receiver (Newport, 125 MHz) was assisted as a detector. In order to maintain stable temperature and pressure, precise feedback system of flow, temperature and pressure was constructed and sample cavity was placed in the system.

As a preliminary result, it was observed that the temperature and pressure were controlled with in 0.1 C and 0.2 mtorr. Transient CRDS signal was also observed as shown in attached Fig. And about 2 % difference of methane signal was observed between reference 2 ppm methane in dry air matrix and air matrix with 50 % RH.

Keywords: Instrumentation, Spectroscopy, Standards
Application Code: Validation
Methodology Code: Near Infrared
Infrared spectroscopy has traditionally been a preferred method for analyzing fuels and lubricants. We will describe a novel liquid sampling device for the analysis of viscous materials that is both fast and accurate and can be applied to many of the ASTM methods developed for analyzing biofuel content, oil additives and conditioning monitoring. The basis of this instrument is a high precision, variable pathlength transmission accessory that can be automatically optimized for an application or even for different components in a single sample. We will present the application of this technique in several areas and discuss the advantages of this approach to the analysis of viscous liquids that are difficult to measure in sealed liquid cells.
The self-assembly of octapeptides derived from human islet polypeptide (hIAPP)22-29 and its two fluorinated derivatives were monitored by vibrational spectroscopy (Raman and IR). These octapeptides (NFGAILSS) which differs only on the absence and presence of fluorine in the aromatic group (phenylalanine) aggregated immediately upon dissolution in aqueous samples. Results from Raman and IR (second derivative) spectra of solid samples showed common peaks among the three octapeptides such as the amide band found around 1675 cm⁻¹. This is due to the common backbone structure possessed by samples before and after self-assembly. In addition, peak differences can also be observed among the three samples which is due to the presence and absence of fluorine.

Keywords: Infrared and Raman, Peptides, Vibrational Spectroscopy
Application Code: Biomedical
Methodology Code: Vibrational Spectroscopy
The development of three dimensional (3D) tissue cultures has been gained big attention by the pharmaceutical companies for drug discovery studies. The 3D cultures are able to accelerate the researches during drug discovery process by creating a greater similarity between the cultured cells and the living organism, and providing a continuously available in vitro disease models leading to more useful data and relevant research on differentiation, drug metabolism, gene and protein expression, general cell function, morphology, proliferation, response to stimuli and cell viability. To obtain molecular information about the cellular responses upon drug exposure, the molecular techniques including immunoblotting, fluorescence labeling based on detection of the caspases, LC3 and p62 protein levels, phosphatidylserin and DNA labeling, and DNA fragmentation are routinely employed. However, these techniques are destructive, high cost and limit the monitoring of long-term effect of drugs on the same sample. The aim of the study is to develop a rapid, cost effective noninvasive, nondestructive, and label-free cell death monitoring technique in 3D tissue cultures for in situ detection of cell death by Raman and surface enhanced Raman spectroscopy. The spheroid cell model was used as a model 3D culture in this study. The spectral changes from intrinsic “finger-print” spectra of anti-cancer drug treated cells showed that this study will provide a new area for monitoring of the cell death in a rapid and cost effective way compared to molecular based techniques.

Abstract Text

Keywords: Raman, Surface Enhanced Raman, Toxicology
Application Code: Clinical/Toxicology
Methodology Code: Vibrational Spectroscopy
The analysis of gases using Raman spectroscopy is difficult because of the low gas density. In previous work we demonstrated 10-20 fold enhanced Raman signals for gas measurements using a silver-coated multipass capillary cell (MCC). The MCC provides enhanced Raman signals by increasing the interaction volume between the laser and the gas inside the cell. The advantages of the MCC are easy alignment and simple construction. In more recent work fiber-optic probes are being used to couple a spatial heterodyne Raman spectrometer (SHRS) to the MCC. The use of a fiber-optic probe makes optical alignment between the MCC and the spectrometer much easier while allowing better light collection. The large acceptance angle, wide field of view of the SHRS, and the absence of a slit allows large optical fibers to be used without loss of light. This in turn allows larger MCCs to be used which should provide improved overall sensitivity. In this paper new types of fiber-optic Raman probes, designed specifically for coupling to an MCC will be described and the performance evaluated. Also, the ability of the SHRS to efficiently collect Raman scattered light from large diameter MCCs will be discussed.
The irradiation of dental caries ("tooth decay") by focused microwave energy (FME) may constitute a novel approach to their non-invasive management. We hypothesize that killing the caries-causing bacteria with FME promotes a local pH shift that is conducive to spontaneous remineralization of lesions. The objective of the present work was to test the hypothesis that microwave irradiated caries preferentially remineralize compared to controls. To test the hypothesis, artificial caries were induced in tooth fragments, which were subsequently exposed to FME. The FME samples and their controls were then exposed to remineralizing solutions. The degree of remineralization of the samples as well as their surface chemistry was determined using FT-Raman spectroscopy, a technique that provides insight into both the surface structure in general, and the distribution and concentration of calcium phosphate species. Given its ease of use and non-destructive nature, FT-Raman compares well to alternate methods of mineralization determination, such as scanning electron microscopy, hardness testing or 3-D imaging. Since remineralization is heterogeneous, the automated spectral collection facility of the FT-Raman instrument was used to obtain detailed surface mapping that provided insight into the distribution of phosphate species. The results of the FT-Raman interrogation showed significant variation in organic and mineral phase concentration, and that FME exposed samples had preferentially remineralized. We concluded that FME of caries may provide an alternate method of treatment, and that FT-Raman mapping provides an easy method for rapidly processing samples for the evaluation of mineralization.

Keywords: Biological Samples, Biomedical, Medical, Microspectroscopy
Application Code: Biomedical
Methodology Code: Vibrational Spectroscopy
Arsine is a key gas used in many semiconductor process systems, and it is essential for manufacturing, and analytical, procedures to meet tight specifications for this product. It is necessary to be able to produce and analyze arsine which has low parts per billion (ppb) levels of moisture contamination to meet the demands of customers. Fourier Transformed Infrared Spectroscopy (FTIR) is capable of consistently providing accurate moisture data down to the low ppb range, and additionally provides real-time data that allows changes over time to be closely observed. This makes FTIR dynamic enough to monitor effects of environmental changes, such as temperature, for finite vessels, as well as introduction of upstream moisture contamination for continuous flows. An MKS FTIR analyzer was used to develop a moisture analysis method for analyzing pure arsine for trace levels of moisture. The method was developed and tested under controlled laboratory conditions at a Matheson Tri-Gas Inc. facility. A certified dry pure arsine vessel was diluted downstream with doped gas of a known moisture concentration. A calibrated moisture generator using a NIST traceable moisture standard was used to dope the dry arsine flow at different concentrations levels ranging from above to below accepted product tolerances. The experiment tested the predetermined parameters of: linearity, repeatability, accuracy, and minimum detection limit (MDL). This study will demonstrate the successful analysis of trace moisture in Arsine and will meet our criteria for success for a measurement capability assessment of this method. The FTIR was able to achieve a 16ppb MDL, an R2 value of 0.9993 for linearity, the relative standard deviation was 1.33% in the repeatability test, and the overall accuracy was greater than 95% (error<±5%) for the measurement of trace moisture in arsine.

Keywords: FTIR, Method Development, Water
Application Code: Quality/QA/QC
Methodology Code: Vibrational Spectroscopy
Cotton fiber consists of natural cellulose I component and its end-use qualities depend on the amount of cellulose deposited during the growth. The term fiber maturity has been used to describe the degree of cellulosic development or the secondary cell wall thickening of fibers. Useful information about fiber maturity is of value to cotton breeders and growers for cotton enhancement and to textile processors for quality control. Undoubtedly, current-in-use cotton maturity measurements can meet the expectations from very differing fiber researchers. However, one of challenges is how to provide accurate maturity information on limited cotton fibers from breeders. To this point, FTIR technique, being structure sensitive and capable of microsampling, was examined as a potential to be applied in the rapid, non-destructive, and routine determination of cotton fiber maturity and crystallinity.

**Keywords:** FTIR, Materials Science, Quantitative, Vibrational Spectroscopy

**Application Code:** Materials Science

**Methodology Code:** Vibrational Spectroscopy
Accurate molar absorptivity values are critical for developing meaningful computer simulations of near infrared spectra. Such models are needed to provide insight into the selectivity, sensitivity, and limit of detection of \textit{in situ} near infrared spectroscopic measurements, particularly for complex samples of biological origin. Molar absorptivity measurements require knowledge of all mechanisms of radiant power loss during a transmission experiment. Besides attenuation by absorption processes, reflective losses at each interface (air/window and window/solution) must be considered. In one common approach, reflective losses are estimated using the Fresnel equations with knowledge of the refractive index for each medium in the optical path. For situations where the sample of interest does not significantly absorb radiation over the targeted wavelength range, reflective loss terms can be eliminated by using the difference between two transmission measurements at two different path lengths.

This research focuses on accurate molar absorptivity measurements for solutes dissolved in aqueous media over near infrared wavelengths. Accurate measures of solute molar absorptivity demand correction for reflective losses, the impact of solvent absorption on reflective losses and light attenuation due to solvent absorption. A general expression is derived that considers the effect of solvent absorption on molar absorptivity measurements for solutes dissolved in an absorbing solvent. Molar absorptivity values determined with this expression reveal relative errors as high as 90\% when solvent absorption is not considered. Magnitude of such errors depends on both path length and wavelength.
In this lecture, we shall review our recent work towards the development of electrochemical imaging of biological cells and tissues. First, we shall present the design and fabrication of soft electrode probes for scanning electrochemical microscopy (SECM), which include microchannels to afford the delivery of chemical effectors or the sampling of solutions in the area of interest. We shall show images of tyrosinase activity on different substrates, for instance for imaging black spots on a banana skin and more important for imaging cancer tissues. We shall also describe how we can perturb the local chemical environment and measure cells’ response both electrochemically or optically.

In the second part, we shall present the working principle of electrostatic spray ionization (ESTASI), which is a contactless pulsed technique recently developed to generate ions from different substrates for mass spectrometry analysis or imaging (MSI). We shall present the coupling of separation techniques such as thin layer chromatography or gel electrophoresis with MS. We shall also present some results for ESTASI-MSI of cells.

Keywords: Bioanalytical, Electrochemistry, Lab-on-a-Chip/Microfluidics, Mass Spectrometry
Application Code: General Interest
Methodology Code: Electrochemistry
We report electrochemical measurements and simulations of the behavior of a thin-layer cell comprising either gold or platinum electrodes separated by 30 to 200 nm of supporting electrolyte. We demonstrate that the electrical fields extending from the electrodes modifies the transport of redox ions, giving rise to new current-voltage responses, and in some instances, completely blocking the redox reactions. The current-voltage response of these electrochemical cells are in semi-quantitative agreement with finite-element simulations based on a Stern-Gouy-Chapman model to account for the electric field extension from the electrode surfaces.
Nanostructured surfaces can be designed with unique optical and physical properties for the fabrication of biosensors, metamaterials and magnetic materials. In this talk we demonstrate a simple low-cost, large-area fabrication method that employs a combination of colloidal lithography and nanoscale electrodeposition to make metallic, semiconductor and polymer nanoring arrays. This nanoscale electrodeposition strategy allows for the independent control of the nanoring diameter, thickness and spacing in the array. These metamaterial surfaces can be used to create simple yet sensitive plasmonic refractive index biosensors, optical materials including dichroic filters and split-nanoring resonators, and magnetic storage materials such as flux closure nanoring arrays. Additionally, related nanofabrication methods are used to make nanowire diffraction gratings and biomimetic nanocone arrays. For biosensor applications, these nanostructured surfaces can be combined with enzymatic and nanoparticle signal enhancement strategies to detect both protein and nucleic acid biomarkers at femtomolar concentrations.

Keywords: Biosensors, Electrochemistry
Application Code: Bioanalytical
Methodology Code: Other
Due to the combination of the world's increasing energy demand and concerns of fossil-fuel combustion induced climate change, there is an imminent need to switch to carbon-free renewable energy sources such as solar. Solar irradiation is diurnal and intermittent, however, making it essential to store a large fraction of the converted energy. For these reasons, photochemical water splitting to generate renewable hydrogen has attracted a lot of attention. Storage and transport of low-density hydrogen gas is problematic, however, making the conversion to higher energy density fuels attractive. One option is the storage of renewable hydrogen as ammonia since it has a relatively high energy density, is carbon-free, and can be potentially be decomposed back to nitrogen and hydrogen with less than 0.1 V (compared to over 1.23 V for water electrolysis) or used in a direct ammonia fuel cell. In practice, however, large overpotentials are required to drive the overall ammonia electrolysis reactions resulting in poor conversion efficiencies. We have therefore been investigating reducing the ammonia electrolysis overpotentials through homogeneous and heterogeneous catalysis. Results of electrocatalytic ammonia splitting in non-aqueous solvents as well as in liquid ammonia at metal and metal oxide surfaces will be discussed. In addition, initial results of the electrocatalytic reduction of N2 will be presented.
In this presentation I will discuss the development of a high-efficiency, dual channel-electrode (DCE) generation-collection system and its application for interrogating redox-active surface-adsorbed thin films. DCE systems consist of two electrodes configured on the base of a microfluidic channel. Under laminar flow conditions, a redox reaction can be driven on the upstream generator electrode, and the products carried by convection to the downstream collector electrode where the reverse redox reaction occurs. One significant outcome of our study is that simple fabrication techniques can be used to prepare DCE systems that have collection efficiencies of up to 97%. This level of efficiency makes it possible to quantitatively measure the charge associated with redox-active thin films interposed between the generator and collector electrodes. This is important, because it provides a means for interrogating species that are not in sufficiently close proximity to an electrode to enable direct electron transfer or electroactive films adsorbed to insulating surfaces. Here, the method is demonstrated by comparing results from this indirect surface interrogation method and direct electroreduction. These experimental results are further compared to finite-element simulations.
The capability to separate and analyze a wide range of proteins in complex systems remains a prime requirement in the biochemical sciences. Protein separations are especially difficult as these large molecules can assume different conformations, association states and amphoteric features presented to chromatographic surfaces. Combining high performance liquid chromatography (HPLC) and ultra high performance liquid chromatography (UHPLC) with mass spectrometry (MS) has proved to be an effective approach for solving difficult problems involving protein analyses. Considerable effort has been made to develop columns for separating proteins with high efficiency for reversed-phase, ion-exchange, hydrophilic interaction liquid chromatography (HILIC) and hydrophobic interaction chromatography (HIC). Even so, many situations still exist where insufficient resolution is available for accurate protein analysis even when high-resolution MS is available. This presentation discusses new approaches for obtaining superior protein separations with columns of highly-efficient silica particles, new techniques in the use of MS-friendly mobile phases and effective methods for changing protein selectivity (band spacings) by column type and organic mobile phase modifiers.
A number of manufacturers now offer a two-dimensional liquid chromatograph instrument. Some of these employ the comprehensive mode, whereby small volumes of a zone exiting the first dimension column are injected into the second dimension column after collection in a sample loop. This process is repeated throughout the separation. The usual case is that the first dimension column is run slowly to give the second dimension separation enough time to fully elute the zone(s) before the next sample is injected.

Another way to do this is to collect narrow fractions of zones and then run them on another column sequentially, perhaps overnight, and then reassemble the chromatographic information. This is referred to as the “offline” method.

Which method is the best use? In some cases this depends on what the goal is – does the analyst only need some zones from the first dimension and then selectively runs this area? Or does the zone crowding (i.e. saturation) dictate that a comprehensive experiment can’t resolve the complexity, for example in a top-down proteomics experiment.

In this presentation we will use theory and experiment to generate guidelines on when the comprehensive experiment can’t keep pace with high saturation samples and when the off-line collection must be performed. In addition, using mass spectrometry to resolve some of the high saturation will be discussed in terms of a compound statistical overlap theory of chromatography/mass spectrometry.

Keywords: Bioanalytical, Liquid Chromatography/Mass Spectroscopy, Statistical Data Analysis
Application Code: Bioanalytical
Methodology Code: Computers, Modeling and Simulation
Column selectivity is determined by various solute-stationary phase interactions. For the case of type-B alkylsilica columns, five such interactions contribute to column selectivity: hydrophobic, steric, ionic, plus hydrogen bonding to acidic or basic sites in the stationary phase. By means of the hydrophobic-subtraction model it is now possible to characterize any reversed-phase column in terms of five related column properties (H, S*, C, A and B). Apart from the case of column hydrogen-bond basicity (B), the remaining column properties H-A are well understood and can be described by specific column properties such as ligand length and density, pore diameter and end-capping. However, the nature of stationary phase sites for hydrogen bonding with acidic solutes is still in question. The present study will review what is known about column hydrogen-bond basicity, which in turn suggests that vicinal silanols are responsible.
Supercritical fluid chromatography (SFC) was first demonstrated some fifty years ago but it has faced a number of highs and lows. Now that some manufacturers have taken it in their hands to produce high-quality robust machines, SFC is presenting a new face to analysts looking for fast, high efficiency, economical and ecological methods. Not only the machines have improved, but the way to practice the method has somewhat evolved with time. Packed columns, identical to those used in HPLC, are now preferred over GC-type capillary columns. This means that SFC has benefitted of the technical progress on silica particles (superficially porous particles and sub-2 µm particles) in the same manner as HPLC. But SFC has the additional advantage of a low viscosity mobile phase, resulting in very high efficiency even with low pressure drops. Also the composition of mobile phase is now considered in a more flexible manner than it used to be, with varied mixtures of carbon dioxide, co-solvents and additives. As a result, the polarity range of the compounds that are amenable to the technique has significantly extended. In this presentation, an overview of the current practice and possibilities offered by this versatile technique will be exposed, with many examples taken from varied application areas (pharma, cosmetics, food science, environment...).

**Keywords:** Chiral Separations, Chromatography, Drug Discovery, SFC

**Application Code:** Other

**Methodology Code:** Supercritical Fluid Chromatography
Many larger pharmaceutical companies have replaced or abandoned high performance liquid chromatography (HPLC) for chiral analysis and semi-preparative chromatography, replacing it with supercritical fluid chromatography (SFC). In the past, chiral analysis in drug discovery was the main application area for SFC, since it is much faster, cheaper to operate, produces much smaller fractions of all organic solvent, and is considered “green”. With recent improvements in instrumentation, particularly lower UV noise, SFC is likely to expand into many other industries, particularly since it is orthogonal to rHPLC.

Surprisingly, most smaller pharma companies continue to use HPLC for these purposes. Similarly, a review of the content of the journal “Chirality” over a years time reveals almost no mention of SFC. A major reason for this difference is the lack of academic training in SFC. There are probably less than 2 dozen SFC’s in academic labs around the world. There is a strong need for both fundamental and applied academic research in SFC.

Keywords: Chiral, HPLC, SFC, Supercritical Fluid Chromatography
Application Code: General Interest
Methodology Code: Supercritical Fluid Chromatography
Sampling and ionization of analyte under ambient conditions are critical for modern mass spectrometry (MS) studies. The sampling size and ionization approach largely determine to which extent a specific MS method can be applied. Recent developments in bioanalytical MS areas, including single cell analysis and MS imaging (MSI) of biological tissues, greatly depend on the advancements in sampling and ionization methods. We are developing a miniaturized multifunctional device, the Single-probe, for multiple research aims.

To fabricate a Single-probe, a dual-bore quartz tube is pulled to a sharp needle (tip size <10 μm), and one fused silica capillary (providing sampling solvent) and one nano-ESI emitter are glued into both bores of quartz needle. A small liquid junction (<10 μm) is formed between two bores at the Single-probe tip for sampling small targets, and the sampled species is immediately ionized by nano-ESI emitter for MS analysis. The Single-probe has been coupled with a Thermo LTQ Orbitrap XL Mass Spectrometer for studies in two different research areas. (1) MS of living single cells. The tip of the Single-probe is small enough to be inserted into living individual cells, and the intracellular compounds are sampled and ionized for direct MS analysis in real-time. A variety of cancer cell lines and anti-cancer drugs have been involved in studies. We have detected cell metabolites, anti-cancer drugs, and cellular species induced by anti-cancer drug treatment. (2) MSI studies: This set-up can be used to sample compounds on surfaces; scan was performed by moving tissue position placed on a XYZ stage system. We have obtained high spatial resolution (<10 μm) under the ambient conditions using the Single-probe MSI device. We have applied our technique to biological tissues and reconstructed the spatial distributions of species of interest on plants or animal tissue slices.

**Keywords:** Bioanalytical, Imaging, Mass Spectrometry, Pharmaceutical

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
Lipids isomers with different C=C bond locations have dramatically different chemical, biophysical, and biochemical properties. Their relative ratios are important indicators for the variations in their biosynthesis pathways, which is highly relevant to the disease study and diagnosis. Our group recently showed that the locations of C=C within a lipid molecule can be determined in a sensitive and confident fashion by coupling Paternò–Büchi (P-B) reaction and subsequent tandem mass spectrometry (MS/MS). The P-B reaction proceeds via [2+2] addition between the UV (254 nm)-excited acetone and a C=C bond of a lipid, forming a product with mass 58 Da higher than the intact lipid. This P-B reaction product can be mass isolated and further subjected to collision-induced dissociation (CID). Collisional activation of the P-B reaction products produces diagnostic fragment ion pair mass separated by 26 Da, which are specific to the C=C bond location within a lipid acyl chain. The above method allows simultaneous characterization of multiple lipid C=C isomers from mixtures. More importantly, the relative intensities of the diagnostic ions can be used to quantify each isomer. The potential and scope of this method is demonstrated with lipid extracts from biological samples (tissues and blood) for simultaneous unsaturated lipid characterization and quantitation.
Photodissociation of peptide ions is one of the fragmentation methods used to tease out peptide sequence and apply it to protein analysis. Peptide ions absorb in the IR and far UV regions that have been extensively used for photodissociation and mass spectrometric analysis. In contrast, peptide ions do not have natural chromophores to absorb in the near UV region. We report two kinds of experiments that allow photodissociation of peptide ions in the near UV region at 355 nm. Experiments of the first kind use artificial chromophores that are introduced into the peptide sequence in the form of photoleucine (L*) or photomethionine (M*) residues. These are L-2-amino-4,4-azi-pentanoic acid and L-2-amino-5,5-azihexanoic acid residues containing the diazirine ring which absorbs light at 350-370 nm and makes the peptide ions amenable to near UV photodissociation. L* and M* are introduced in the peptide sequence by standard solid-phase synthesis, or they can be incorporated into proteins by expression where they replace the natural L and M residues. The other kind of experiment does not require a photoactive label. It combines electron-transfer dissociation (ETD) with photodissociation, where ETD generates a new chromophore to be probed by photodissociation at 355 nm. Examples of both methods will be presented.
There is evidence that epimerization, which occurs when a single amino acid converts to the D-configuration, is an important spontaneous post-translational modification (PTM) that occurs as a function of aging. Epimerization leads to no change in mass and it is significantly more difficult to detect than the other PTMs, which may explain why epimerization has received significantly less attention. Quantitative analysis of peptide epimers is typically carried out by calculation of an R value that corresponds to the degree of difference between the two fragments that change the most in the MS2 spectra obtained from each isomer. Recent work has demonstrated that radical directed dissociation (RDD) yields the highest R values for epimer detection and has the advantage of the greatest flexibility in terms of charge state selection.

In the present work, we describe characterization of isomeric PTMs in sheep crystallins extracted from the eye lens. The results from both RDD and CID on LC separated peptides were combined to improve isomer identification. A short exclusion time and a target peptide mass list were used to ensure that each peptide was examined multiple times to allow for comparison of the relevant tandem mass spectra. Three crystallin proteins (A-, B-, and B3-crystallin) were identified from the ovine database with excellent sequence coverage. Several additional proteins that are predicted to be associated with crystallin are also identified from the newly released sheep genome. Many previously uncharacterized sites of isomerization for crystallins were identified. The results illustrate that the greatest degree of isomerization and epimerization occurs in the disordered N-terminal and C-terminal regions of A- and B-crystallin, which are abundant and important proteins in the lens that function as chaperones and also serve as structural elements.

Keywords: Amino Acids, Chiral, Protein
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Native mass spectra of proteins and protein complexes often contain narrow charge-state distributions and wide peaks, which makes assigning charge states challenging and can limit the accuracy of native mass spectrometry experiments. These complications are exacerbated when samples contain mixtures of proteins or oligomeric states. Here we use Cation to Anion Proton Transfer Reactions (CAPTR) reactions to generate charge-reduced product ions. CAPTR is analogous to ion/ion proton transfer reactions pioneered by McLuckey and coworkers to aid in the analysis of peptides and other analytes ([i]Chem. Commun.[/i] [b]2013[/b], [i]49[/i], 947–965). Using CAPTR, we have detected the lowest charge state below [i]m[/i]/[i]z[/i] 100 000 for a series of protein complexes ranging from 12–468 kDa. Charge states are assigned unambiguously using the additional charge-reduced peaks, which enables accurate oligomeric state and mass assignments. Additionally, we demonstrate the ability to separate proteins in a mixture by reacting ions selected from a congested [i]m[/i]/[i]z[/i] region containing contributions from multiple protein complexes. Using this approach, we observed a >2000 fold increase in the resolution of two components from that mixture. The improved resolution of the CAPTR products is attributable to selecting precursor ions of similar [i]m[/i]/[i]z[/i] but different [i]z[/i]; thus, the resolution increases following each proton transfer reaction. Lastly, collision cross sections of several protein complexes were determined. These results show a reproducible, but subtle range of collision cross sections (–3% to +3%) for protein complexes as a function of charge state. Our results show that CAPTR is an efficient method to manipulate the charge states of native-like ions, which we use to accurately assign charge states, separate complex protein mixtures, and probe the relationship between charge state and protein structure.

Keywords: Electrospray, Mass Spectrometry, Tandem Mass Spec, Time of Flight MS
Application Code: Genomics, Proteomics and Other ‘Omics
Methodology Code: Mass Spectrometry
Intense research effort in low-temperature (or cold) atmospheric plasma application in bioengineering led to foundation of a new field, plasma medicine. Low temperature plasmas (LTP) produce chemically reactive species including reactive oxygen species (ROS) such as O, O2-, and OH and reactive nitrogen species (RNS) such as NO and NO2. These species are known to have biological effects on prokaryotic and eukaryotic cells, such as the peroxidation of lipids and proteins. In addition, they are known to trigger signaling pathways in biological cells. Although LTP has been shown to be lethal to bacteria, under some conditions it appears to cause little damage to mammalian cells. Having different structure and morphology than prokaryotes, mammalian cells exhibit different responses to physical and chemical stresses. The ability of LTP to kill bacteria and to accelerate the proliferation of specific tissue cells opened up the possibility to use plasma for the healing of chronic wounds such as diabetic ulcers. Tens of thousands of amputations occur every year in the US alone because of the inability of present medical methods to heal chronic wounds.

The most recent research area of plasma medicine is the interaction of LTP with cancer cells. It has been demonstrated by several investigators that LTP can induce apoptosis (programmed cell death) in various cancer cell lines. In addition, LTP treatment affects preferentially the cell cycle of cancer cells. This opened up the possibility that LTP could be the basis of a new cancer therapy. So far both in vitro and in vivo experiments have been carried out with encouraging level of success, but no clinical trials have been attempted. The above mentioned biomedical applications seem to usher a new transformational approach to healthcare based on LTP technology. In this presentation a review of the research achievements accomplished in the last decade will be presented.

Keywords: Biomedical, Plasma Emission (ICP/MIP/DCP/etc.)
Application Code: Biomedical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Over the past two decades, there has arisen increasing interest in liquid-electrode-supported glow discharges as alternative sources for atomic optical emission spectrometry (OES). Among these sources, the solution-cathode glow discharge (SCGD) has received substantial attention due to the many advantages it offers over more conventional plasma sources for atomic spectrometry, such as the inductively coupled plasma (ICP). Simple and inexpensive to construct, the SCGD operates in the ambient atmosphere (requiring no compressed gases), samples directly from a flowing stream of sample solution (eliminating the need for a nebulizer), is driven by low, direct-current power (typically <70 W), yields surprisingly uncluttered emission spectra, and produces detection limits that are comparable to or better than those obtained with radially viewed ICP-OES.

Many past studies of the SCGD have revealed that elemental and molecular emission are not spatially homogenous throughout the source, but rather conform to specific zones within the discharge. Exploiting this inhomogeneity can lead to improved analytical performance if emission is collected only from the regions of the discharge where analyte species emit strongly and background emission (from continuum, elemental and/or molecular sources) is lower. Here we will present a comparative study of the effects of spatial selection on the analytical aspects of the SCGD, with emphasis on detection limits, precision and linear range. Use of spatial selection to reduce or eliminate matrix effects will also be evaluated.

**Keywords:** Atomic Emission Spectroscopy, Atomic Spectroscopy, Elemental Analysis, Plasma Emission (ICP/MIP/

**Application Code:** Environmental

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
Plasma-based ambient desorption/ionization mass spectrometry (ADI–MS) has attracted considerable attention in many fields because of its capacity for direct sample analyses. We have developed a high-power pulsed microplasma jet as a plasma desorption/ionization source. In the plasma jet, a micro hollow cathode discharge is generated in a small hole (500 μm in diameter) using a pulsed high-power supply. This system can realize a maximum power density of 5 x 10^8 W/cm^3. The measured electron number density and excitation temperature were 3.7 x 10^15 cm^-3 and 7,000 K, respectively. But the gas temperature was less than 60[degree]C because it is a non-thermodynamic equilibrium plasma. And the plasma potential was made almost zero. Therefore, the plasma does not give thermal and electric discharge to the target. Thus, we can apply it directly to human skin such as shown in the figure. To demonstrate the analytical capacity of ambient desorption/ionization mass spectrometry, the plasma was applied to direct solid sample analysis of the active ingredients in pharmaceutical tablets. Caffeine, acetaminophen, ethenzamide, isopropylantipyrine and ibuprofen were successfully detected. Application to living tissue was also demonstrated, and isopropylantipyrine on a finger was successfully analyzed without damaging the skin. The limits of detection (LODs) for caffeine, isopropylantipyrine and ethenzamide were calculated, and LODs at the picogram level were achieved. We applied for organic mass spectrometry of Chemical Warfare Agents (CWA) for strengthen counter-terrorism for such as Tokyo Olympic 2020. To evaluate the analytical performance, VX of 1 μg on the Teflon plate was directly irradiated by air plasma and analyzed by using the mass spectrometer. As a result, the protonated VX was successfully detected with low fragmentation and LOD of VX was 0.13 pmol. The analytical results of other CWA such as GA will be presented.

Keywords: Mass Spectrometry, Medical, Plasma, Sampling
Application Code: Biomedical
Methodology Code: Mass Spectrometry
Glow discharge plasmas have a long-standing analytical history as large, stand-alone laboratory scale instruments. In contrast the physical scaling permits micro-plasma operation at atmospheric pressure while reducing power requirements, thereby permitting mobile analysis. The energetic species in a non-thermal, i.e. “cold” plasma dissociates species of interest and electronically excites the elemental constituents. The atomic emission spectrum serves to identify the compound and ideally its molecular composition with intensity corresponding to concentration. For some operational regimes and species, emission from OH*, CH* and often C2* diatomic radicals is also produced. Such emission further aids compound detection and identification. Detection and identification of acetone, ethanol, heptane, toluene, and nitrobenzene is demonstrated. Limits of detection extend to parts-per-billion levels for some species such as nitrobenzene. Results will be shown for differentiation of classes of organic compounds such as alkanes, aromatics and oxygenates. Application for C/H ratio measurements of soot and other carbonaceous materials will also be presented.

Keywords: Aerosols/Particulates, Analysis, Atomic Emission Spectroscopy, Environmental
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The liquid sampling-atmospheric pressure glow discharge (LS-APGD) holds a unique position among the other atmospheric pressure glow discharges as it has demonstrated abilities in atomic emission, elemental mass spectrometry, molecular mass spectrometry, and ambient desorption mass spectrometry. These base capabilities are multiplied as the sample forms that are amenable to analysis include liquids, bulk solids, solution residues, and aerosols generated by laser ablation. This versatility is affected on a very small platform, with minimal supporting utilities. Specifically, the plasma is ignited between an electrolytic solution that is introduced at flow rates of 10-200 microliters/min at discharge currents of <60 mA, and d.c. powers of <50 W. The counter electrode can take the form of a solid metal, or a hollow metal capillary, which allows the introduction of a counter flow of sheath gas or the entrainment of aerosol sample material. The discharge volume is set by the electrode gap, but is usually on the order of 1 cubic millimeter. As such, the power densities realized are greater than 10 W/cubic millimeter; a factor of 100-times greater than the ICP. In this presentation, we will describe the design and fundamental operational aspects of the LS-APGD as they pertain to the various spectroscopic sampling modes. The versatility of the source will be graphically demonstrated as the microplasma is converted between an elemental/isotopic ionization source, a source for direct speciation of organometallic compounds, and an ambient desorption MS source in a matter of minutes. It is believed that the LS-APGD microplasma holds a unique place among its peers and warrants investigation across many fields of chemical analysis.

Keywords: Aerosols/Particulates, Atomic Spectroscopy, Elemental Mass Spec, Mass Spectrometry
Application Code: General Interest
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Bioanalytical Applications of Nanofluidics

Polymer Nanofluidic Devices for DNA Analysis

Novel nanofluidic platforms for the analysis of single DNA molecules will be discussed with applications including single-molecule electrophoresis and searching for sequence variations in double-stranded DNA such as methylation patterns or drug-induced abasic sites. The nano-scale channels are produced in thermoplastics using Nanoimprint lithography (NIL) from resin stamps and Si masters. The masters are made using a combination of conventional optical lithography (microchannels) and focused ion beam milling (nanochannels). Thermoplastics can serve as viable alternatives to glass and quartz for nanofluidic devices because of their diverse surface chemistry, optical transparency and the ability to be manufactured in a high production mode using NIL. NIL has proven to be successful in patterning structures to sub-10 nm dimensions with the ultimate resolution determined by the minimum feature size associated with the imprinting tool. In fact, we have made channels as small as 18 nm (width x depth) using NIL. In this presentation we will discuss the electrically driven transport dynamics of both small (deoxynucleotides monophosphates, dNMPs) and large (DNAs) molecules traveling through thermoplastic nanochannels. The effects of scale (i.e., cross-sectional area of polymer nanochannel), electrical double layer overlap producing non-plug-like flow profiles, surface chemistry, surface charge, nano-scale surface roughness and electroosmotic flow (EOF) will be discussed and the effects of these parameters on separation efficiency for nano-scale electrophoresis presented. Data will be also be shared on detecting the presence of DNA damage in genomic DNA by labeling the damaged (abasic) sites specifically with a fluorescent dye. Stretching the DNA in a nanochannel allows for direct reading of the location and frequency of these damaged sites to infer drug efficacy.

Keywords: Biomedical, Biotechnology, Capillary Electrophoresis, Electrophoresis
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Thin membranes are a compelling structural element for the fabrication of devices to deliver unique nanoscale sensing and manipulation capabilities. I will discuss two silicon nitride membrane-based devices: the first, a fluid cell with <100nm-high flow channel for transmission electron microscopy (TEM) studies of liquid samples, and the second, a nanopore-based electronic single-molecule manipulation and sensing platform.

TEM has proven to be a tremendously powerful tool for revealing the nanoscale structure of fixed samples, but the ability to directly probe native structures in solution and to probe solution structural dynamics has remained a compelling but challenging goal. We have developed a nanofluidic sample cell designed to unlock the full potential of TEM for the study of samples in solution. Key to the approach is the ability to confine and flow thin layers of liquid between thin, so-called electron-transparent windows. I will present high resolution TEM movies of nanoparticles in solution and discuss the implications of this imaging and fluidic handling capability.

Nanopores are nanometer-scale channels fabricated into thin membranes. When properly configured, they can be used for single-molecule-sensitive studies of intermolecular interactions by force spectroscopy, and for chemical sensing in solution by resistive pulse sensing. I will discuss methods to fabricate, characterize and enhance nanopores for such single-molecule studies. Particular emphasis will be placed on surface chemical modifications of silicon nitride membranes to control pore size, surface charges and surface functional group presentation.

Funding from the National Science Foundation, Insight Nanofluidics, Inc., and the University of Rhode Island is acknowledged.
We use isotachophoresis (ITP) to achieve fast and specific analyses of molecular targets in complex mixtures. We use ITP to preconcentrate and purify; molecular recognition for specificity; and ITP to control and increase the rate of chemical reactions between molecular probes and target macromolecules. ITP is an electrophoresis technique that uses two buffers which include a high mobility leading electrolyte (LE) and a low mobility trailing electrolyte (TE). Sample species with mobilities bracketed by those of the LE and TE focus into the TE-to-LE interface. For trace sample concentrations, multiple species focus in so-called peak mode wherein multiple analytes mix and strongly overlap within an order 10 µm wide ionic concentration shock wave. This co-focusing mixes target species and preconcentrates them to accelerate reactions. We have integrated DNA and RNA extraction with sequence-specific quantitation using a variety of mobile and immobile cDNA probes. We preconcentrate target and probe molecules by >10,000x into select reaction volumes ranging from 1 to 100,000 pl. We achieve hybridization reactions which would normally take 4 days in 30 seconds. We have shown specific and sensitive detection of target sequences in order 5 minutes with little or no off-chip sample preparation, and without target amplification.

Keywords: Bioanalytical, Biological Samples, Biosensors, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Single pores in resistive-pulse technique have been successfully used for the detection of cells, viruses, particles, and even molecules such as DNA and proteins. We have investigated application of pores with undulating opening diameter for the detection of particles and characterization of their physical properties. The resistive pulses generated by polymer spheres passing through these pores had a repeatable pattern of large variations corresponding to these diameter changes. We showed that this pattern of variations enabled the unambiguous resolution of multiple particles simultaneously in the pore, that it could detect transient sticking of particles within the pore, and that it could confirm whether any individual particle completely translocated the pore. These results have practical importance for increasing the speed of resistive-pulse sensing, optimizing the detection of specific analytes, and identifying particle shapes. We also showed pores with undulating opening diameter developed local pressure drops, which were sufficiently large to probe mechanical properties of passing objects. Application to hydrogels as well as biological cells will be discussed.
Virus assembly is a highly coordinated process in which hundreds of subunits react to form complex and symmetric particles. Single-particle methods that examine the mechanisms by which capsid reactions occur are lacking, and measurement of low-abundance intermediates on path to forming complete particles is particularly challenging. We are using resistive-pulse sensing as a label-free, nondestructive technique to characterize the assembly of Hepatitis B Virus Cp149 dimers into \( T = 3 \) and \( T = 4 \) symmetry capsids in 1 M NaCl. This single-particle method permits real-time detection of both capsid formation and intermediate depletion and has sufficient sensitivity to monitor assembly at dimer concentrations as low as 50 nM, well below the pseudo-critical dimer concentration. Assembly reactions below, near, and above the pseudo-critical dimer concentration reveal three distinct regimes of assembly. Below the pseudo-critical dimer concentration, where the early steps of assembly are rate- and product-limiting, the ratio of \( T = 3 \) to \( T = 4 \) capsids increases with decreasing dimer concentration. In contrast, far above the pseudo-critical dimer concentration, incomplete particles assemble rapidly, become kinetically trapped, and slowly anneal into \( T = 4 \) capsids. These single-particle measurements have provided new insights into assembly paths and energetics.
The complexity of biological samples poses a major challenge for reliable compound identification in both NMR and mass spectrometry (MS), a problem that is of major concern in the field of metabolomics. The presence of interfering compounds that causes additional peaks to appear in the spectrum can make interpretation and assignment difficult. To address this issue, we have proposed a new approach we call RANSY[1], which extracts the 1H signals related to the same metabolite based on peak intensity ratios. The RANSY algorithm calculates the ratio of the peaks in the NMR spectrum with a peak of interest called the “driver peak,” and then divides that ratio by its standard deviation across the sample set. We have recently extended this approach to mass spectrometry as well (RAMSY)[2], which facilitates identification of unknown compounds in complex MS spectra. RAMSY works a very similar principle that, under a given set of experimental conditions, the abundance/intensity ratios between the mass fragments from the same metabolite are relatively constant. Therefore, the quotients of average peak ratios and their standard deviations, generated using a small set of MS spectra from the same ion chromatogram, efficiently allow the statistical recovery of the metabolite peaks and facilitate reliable identification. RAMSY has been applied to both GC-MS and LC-MS/MS data to demonstrate its utility. The performance of RAMSY is typically better than the results from correlation methods. RANSY and RAMSY promise to improve the process of unknown metabolite identification for NMR and MS users in metabolomics and/or other fields.

References


Keywords: Bioanalytical, Chemometrics, Mass Spectrometry, NMR
Application Code: Bioanalytical
Methodology Code: Data Analysis and Manipulation
We develop a novel peak detection algorithm for the analysis of comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-TOF MS) data using normal–exponential–Bernoulli (NEB) and mixture probability models. The algorithm first performs baseline correction and denoising simultaneously using the NEB model, which also defines peak regions. Peaks are then picked using a mixture of probability distribution to deal with the coeluting peaks. Peak merging is further carried out based on the mass spectral similarities among the peaks within the same peak group. The algorithm is evaluated using experimental data to study the effect of different cutoffs of the conditional Bayes factors and the effect of different mixture models including Poisson, truncated Gaussian, Gaussian, Gamma and exponentially modified Gaussian (EMG) distributions, and the optimal version is introduced using a trial-and-error approach. We then compare the new algorithm with two existing algorithms in terms of compound identification. Data analysis shows that the developed algorithm can detect the peaks with lower false discovery rates than the existing algorithms, and a less complicated peak picking model is a promising alternative to the more complicated and widely used EMG mixture models.

Keywords: Bioinformatics, Identification, Mass Spectrometry, Metabolomics, Metabonomics
Application Code: High-Throughput Chemical Analysis
Methodology Code: Chemometrics
Bioinformatics: Metabolite Identification and Quantification

Discriminating Precursors of Common Fragments for Untargeted Metabolomics

The goal of large-scale metabolite profiling is to compare the relative concentrations of as many metabolites extracted from biological samples as possible, however, identifying the structures of the thousands of ions detected by QTOF and Orbitrap mass spectrometers is prohibitively time intensive. Here we describe a scheme for using QqQ mass spectrometers to perform large-scale metabolite profiling to facilitate high-throughput structural characterization. First, we identify common fragmentation products from METLIN's experimental MS/MS data. Then, we model the likelihoods of each precursor in METLIN producing each common fragmentation product. From these likelihood estimates, we pick ensembles of common fragmentation products that minimize our uncertainty about metabolite identities and can therefore be used to build MRMs for thousands of compounds from the METLIN database. We demonstrate the application of our approach by characterizing metabolic alterations associated with inflammation in astrocytes and oligodendrocytes.

Keywords: Bioinformatics, Biological Samples, Liquid Chromatography/Mass Spectroscopy, Metabolomics, Meta
Application Code: High-Throughput Chemical Analysis
Methodology Code: Mass Spectrometry
The stable isotope assisted metabolomics (SIAM) uses stable isotope tracers (e.g. 2H, 13C and/or 15N) to support studies of biochemical mechanisms. It is able to discern the originating pathways and effectively measure the levels of metabolites associated with a particular pathway, if multiple pathways result in the same product metabolite. We have developed a bioinformatics platform to process LC-MS and GC-MS experiment data acquired from SIAM projects. The developed bioinformatics platform includes capability of mass spectrum peak picking, deconvoluting overlapped isotopic peaks of isotopologues, metabolite assignment, cross sample alignment, normalization, metabolite quantification, and correlation network analysis. The metabolite assignment in the LC-MS data is achieved based on m/z value and the isotopic peak profile matching, which by mass spectrum matching and retention index filtering in analysis of GC-MS data.

SIAM follows the fate of the heavy atoms and their incorporation into a multitude of metabolites produced from the labeled tracer. This not only helps identify and quantify isotopologues, but it also can determine the position of the labeled atom in a molecule, leading to exact biochemical pathway assignment. Our developed bioinformatics platform has been validated using mixtures of compound standards, spiked-in experiments, and further applied to multiple biological experiments.
The NIH West Coast Metabolomics Center at UC Davis employs accurate mass GC-QTOF MS and reverse phase as well as HILIC UPLC-QTOF MS platforms to screen for metabolic differences in blinded human cohort samples, for example in lung cancer or cardiovascular studies. Comprehensive analyses of these samples identifies over 150 primary metabolites and more than 300 individual complex lipids in lipidomic screens, in addition to specific metabolites such as S-adenosylmethionine, 1-methylnicotinamide, trimethylamine-N-oxide, carnitines, betaines or other polar cationic metabolites. While our focus is to reveal metabolic dysregulation that precedes or informs about human diseases, human cohort studies benefit from information that can further stratify or constrain groups of subjects and that could complement tools such as Food Frequency Questionnaires or co-medication information. Untargeted metabolomics with comprehensive mass spectral fragmentation analysis can yield such data. We give examples how the NIH West Coast Metabolomics Center found dozens of pharmaceutical agents in human lung tissue as well as in blood plasma of larger cohort studies. We highlight how the general metabolomics workflow helps detection and unambiguous identification of xenobiotics such as food components, pharmaceuticals as well as illicit drugs. We regard these results as significant validation towards the concept of comprehensive exposome analysis.

**Keywords:** Bioinformatics, Data Analysis, Mass Spectrometry, Metabolomics

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
Carbon materials have been used over the years in energy storage and conversion, chemical analysis and separations. The widespread interest in carbon stems from its low cost, mechanical strength, chemical stability in a variety of environments and diversity of surface chemistry. Nonetheless, advances in the fundamental understanding of the structure of electrified interfaces formed at various carbon materials and how this structure affects adsorption, charge-transfer kinetics and redox reaction mechanisms remain to be gained. This is particularly true for the novel sp3 (diamond) and sp2/sp3 (tetrahedral amorphous carbon) electrodes. A more complete understanding of structure-property relationships will serve as the foundation for the next-generation of electrochemical sensors and detectors with these materials.

We are developing an improved understanding of structure-property relationships of boron-doped nanocrystalline diamond and nitrogen-incorporated tetrahedral amorphous carbon (ta-C:N) thin-film electrodes. ta-C:N is a composite material consisting of sp2 and sp3-bonded carbon. Impurities can be incorporated during growth further adding to their complex structure. These films typically possess 40-60% sp3-bonded carbon. It has been widely used as a protective coating due to its hardness, high wear resistance and low coefficient of friction. The growth temperature for ta-C is usually from 25 to about 100 oC. This means that non-traditional materials, such as plastics, can be used as substrates for deposition. ta-C films generally need to be grown thin (100’s of nanometers) in order to minimize internal stress. Stressed films tend to delaminate from a substrate. We will report on the characterization and basic electrochemical properties of these new electrode materials and highlight some electroanalytical uses (FIA-EC) such as for the detection of the drug, propranolol.

Keywords: Electrochemistry, Electrodes, Flow Injection Analysis, Materials Science
Application Code: Materials Science
Methodology Code: Electrochemistry
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**Abstract Text**

There has been recent interest in exploring the use of various nanomaterials as adsorbent media for chromatography; among these are the carbon-based nanoparticles carbon dots (C-dots) and nanodiamonds (NDs). We have developed strategies to modify the surface of silica materials with such nanoparticles. Both, silica and monolithic structures have been used as support materials. Of particular interest have been the ND-modified silicas. Nanodiamonds having an oxidized or a hydrogenated surface were attached to silica. The degree of hydrogenation on the NDs can be controlled by manipulating experimental parameters. After physicochemical characterization, we explored the adsorptive characteristics of these materials using liquid chromatography (LC) and supercritical fluid chromatography (SFC). The initial findings suggest that non-hydrogenated ND-modified silica provided ion exchange sites as well as hydrophilic interactions while the hydrogenated ND-modified silica provided more hydrophobic interaction. In this occasion, we will present details of the NDs attachment to silica materials, characterization, as well as the findings of our chromatographic testing.

**Keywords:** HPLC Columns, Liquid Chromatography, Modified Silica, Nanotechnology

**Application Code:** Materials Science

**Methodology Code:** Liquid Chromatography
Fluorescent carbon nanodots (FCNs) have recently received significant attention because of their attractive properties: chemical inertness, biocompatibility and low toxicity. They are considered as promising materials for bioimaging, photocatalysis, sensing, and photovoltaic applications. The confined sp2-carbon conjugation surrounded by chemical functional groups can produce bright, photostable, and tunable photoluminescence. While research has been actively conducted to promote brightness and modulate the optical properties of FCNs, the fundamental understanding of detailed emission mechanisms and structure-property-function relations is still incomplete. Our recent progress in controlling the structure and chemical functionalities of FCNs will be presented. For the optical characterization of carbon dots, single molecule and ensemble spectroscopies were successfully carried out to unveil complicated photophysics and multi-chromophoric nature of FCNs. The application of variously functionalized FCNs for the imaging of cancer cells and their biocompatibility will be also presented.

**Keywords:** Biomedical, Biosensors, Environmental Analysis, Imaging

**Application Code:** Biomedical

**Methodology Code:** Fluorescence/Luminescence
A sputtered nanocarbon film electrode was used to measure some biomolecules. The film was formed by using the electron cyclotron resonance (ECR) sputtering method that provided a nanocrystalline sp\(^{2}\) and sp\(^{3}\) mixed bond structure with an atomically flat surface (surface roughness of 0.05-0.1 nm). This film electrode has excellent properties including a wide potential window, low background current and little surface fouling while maintaining relatively high electrode activity for various biomolecules. These characteristics allow the detection of biomolecules with higher oxidation potentials. For example, this nanocarbon film (sp\(^{2}\)/(sp\(^{2}\)+sp\(^{3}\)) ratio=0.6) electrode can measure all the DNA bases (including the DNA base derivatives e.g., 5'-methylcytosine and 8'-hydroxy 2'-deoxyguanosine) more quantitatively than conventional carbon-based electrodes. Moreover, due to their good electrochemical stability, these sp\(^{3}\)-containing carbon electrodes are suitable for long-term analysis including as the electrode of an HPLC detector for detecting various biological compounds. We also used the nanocarbon film electrode to detect some biomolecules with a low concentration such as lipopolysaccharide (LPS). The nanocarbon film electrode was modified with poly-[epsilon]-lysine ([epsilon]-PL) with a high affinity to LPS. LPS was captured on the modified electrode, and then ferrocene labeled polymyxin B (FcPMB) was captured on the LPS adsorbed electrode via the LPS-PMB affinity interaction. The adsorbed FcPMB provided an amplified response with Fe\(^{2+}\) ions, and the current response was dependent on the amount of captured LPS (LOD=2.0 ng/mL). This was due to the efficient accumulation of the obtained current for LPS and the very low noise made possible by the ultraflat surface. Here, we will present the basic properties of the sputtered nanocarbon film and its applications to the electrochemical detection of some biomolecules.
New forms of carbon as electrodes have open new doors in protein electrochemistry. We have focused on the electrochemistry of insulin using carbon nanotubes as a biosensor, and on the photoelectrochemistry of photosystem I protein complexes, first on carbon nanotubes, but then moving to graphene and reduced graphene oxides.

Direct electrochemical detection of insulin provides sensitivity and rapid analysis times to enable continuous real-time measurements. While previous electrochemical sensors have been used to detect insulin, the limited stability and lifetimes of the electrode films leads to loss of insulin sensitivity. We present an insulin sensor for electrochemical detection in a microfluidic device based on direct oxidation at a MWCNT/Dihydropyran composite film. We have successfully adapted this sensor for the microfluidic chamber of the multianalyte microphysiometer and recorded the response of isolated murine islets to glucose stimulation, as well as nutrient stimulation. The increase in concentration of insulin detected for islets is much higher than the limit of detection and as high as 100 micromolar for the sensor because of the confined volume of the microfluidic chamber and the buildup of insulin molecules during a stop flow period.

Photosystem I (PSI) is one of the primary membrane protein complexes that drive photosynthesis. Our electrochemical interface with graphene electrodes incorporates photosystem I in applied photosynthetic systems. Recently, we developed a completely organic, transparent, conductive electrode using reduced graphene oxide (RGO) on which a multilayer of PSI could be deposited. The resulting photoactive electrode demonstrated current densities significantly higher than a graphene electrode modified with a monolayer film of PSI. We have created the first all carbon based photovoltaic by replacing ITO, semiconductors, and metals with graphene and reduced graphene oxides.

Keywords: Chemically Modified Electrodes, Electrochemistry, Electrode Surfaces, Protein
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Painting a Fresco with Giotto #3 (Indianapolis Museum of Art, #2009.466) by contemporary designer Fernando Brizio comprises a white vase pierced with Giotto-brand felt tip markers. The bleeding of the pen inks creates a playful polka dot surface of solid circles or concentric dye rings around each marker. The vase was newly acquired for a 3 venue exhibition, but returned to the museum badly faded, with some of the ink dots having nearly vanished. Conservation scientists were asked to explore the cause of the fading and to prepare future exhibition and handling guidelines based on the object’s measured lightfastness.

In-situ microfade testing of the inks revealed that many of the colorants are still prone to rapid color loss. Communication with the manufacturer of the markers confirmed that they are student-grade and not intended to have high lightfastness, but rather to be safe and easily cleanable. The identification of the inks from the pens by Raman microspectroscopy proved challenging. Despite acquiring high quality data from the dried ink, only two dyes could be positively identified based on current spectral databases. Analysis of the inks from new Giotto markers was undertaken using HPLC-PDA-MS. This led to the identification of 8 of 9 dyes detected in the 30 different pens: Acid Violet 17, Brilliant Blue FCF, Acid Red 18, Acid Red 14, Acid Red 52, Food Sunset Yellow, tartrazine, and erythrosine. Genuine examples of these dyes were characterized by Raman spectroscopy, confirming that the dyes in the pens from the vase and the new pens contained the same colorants.

The artist was contacted regarding the fading issue, about which he was already aware. Future interviews will record Brizio’s material choices, his thoughts on the status of the IMA piece and whether it still achieves his vision, and how he views the longevity of his work in general. This project highlights the urgency of characterizing the fading rate of potentially fugitive artworks.

Keywords: Analysis, Art/Archaeology, Liquid Chromatography/Mass Spectroscopy, Raman
In the 1960s, the LA art scene blossomed, transforming the city into an international art capital. One of the first truly indigenous and original LA art currents was the “LA Look.” The term refers to loosely affiliated group of Los Angeles artists who used novel materials and processes, often borrowed from different industries, to create a body of work characterized by pristine surfaces, and bright, sensuous colors.

The Getty Conservation Institute has undertaken in recent years a major study into materials and technologies utilized throughout the 1960s and 1970s by artists such as Peter Alexander, Robert Irwin, Craig Kauffman, John McCracken, Helen Pashgian and De Wain Valentine. Because the typically pristine surfaces of the LA Look objects often prohibit any sampling, an important part of the project has been the use of a portable ATR instrument with a curved ATR measuring head for rapid, non-invasive, in situ analysis. Although contact with the object is required with this instrumental setup, the force exerted during measurement is minimal and in most cases extremely high quality FTIR spectra were obtained without leaving any mark on these typically delicate surfaces. However, when possible – typically when pre-existing damages were present – sampling was performed and a more traditional array of analytical techniques used, from microscopic observations to Raman microscopy to identify synthetic organic pigments and py-GCMS to obtain detailed information on the chemistry of the organic media used by the artists.

This paper will present the findings of the project to date and detail the use that LA artists made of synthetic materials, in particular automotive paints and polyester and acrylic plastics. It will also briefly introduce the conservation challenges presented by these objects and how science can contribute to build a range of treatment options through ongoing research on adhesives and repair methods for translucent plastics.

**Keywords:** Art/Archaeology, Polymers & Plastics, Portable Instruments, Pyrolysis

**Application Code:** Art/Archaeology

**Methodology Code:** Portable Instruments
Surfactant migration to acrylic emulsion paint surfaces has been anecdotally and experimentally linked to a wide variety of surface phenomena and conservation challenges on these types of surfaces. Increased soiling, changes in gloss and color, blanching or ‘bloom’ are often cited as common surface aesthetic effects. But as well, mechanical effects such as changes in elastic modulus and Tg at paint surfaces that have accrued surfactant moieties are also of concern with these types of artist’s media. Environmental conditions play a role in surfactant migration and segregation. There appears to be a clear relationship between both temperature and relative humidity (RH) in the ambient environment around acrylic paint films and the accumulation of surfactant at these surfaces. Surfactant hydration and de-hydration under varying temperature and RH conditions appears to affect both the ultimate partitioning of the surfactant at the paint-air interface, as well as the inherent size, aggregation tendencies, and solubility of surfactant in the bulk paint binder. In the present study three-dimensional (3D) microscopy and quartz crystal microbalance with dissipation (QCM-D) were used to add to and reinforce current understanding of the physical and mechanical changes to acrylic paint films with temperature and RH. The migration of surfactant at the film surface was studied using desorption electrospray ionization-mass spectrometry (DESI-MS) and attenuated total reflectance Fourier-transform infrared (ATR-FTIR) microscopy. Electronic speckle pattern interferometry (ESPI), as a method for examining, in virtual real time, the mechanical changes in these paints as a function of the ambient environment around them will also serve to underscore the highly responsive nature of surfactant accumulation on these paint surfaces.

**Keywords:** Art/Archaeology, Imaging, Mass Spectrometry, Paint/Coatings

**Application Code:** Art/Archaeology

**Methodology Code:** Mass Spectrometry
Chemical Issues with Contemporary Art

Analyzing an Artist's Use of Modern Metals and Finishes with Portable Instrumentation

Although he rejected being categorized as a Minimalist, the American artist Donald Judd (1928-1994) is recognized today as one of the leading figures of the 1960’s movement called Minimalism. He created a large body of work that spans the two-dimensional and three-dimensional spatial range, and pioneered the utilization of materials designed for industrial use and had them executed by fabricators of various trades. Judd’s artistic approach opened new venues for art, but also presented unusual challenges for conservation science.

More often then not, the pristine, uniform surfaces of Judd’s works prohibit customary sampling practiced for traditional art. Collecting the tiniest sample would cause permanent damage to these works, hence the removal of samples for analysis is generally severely limited, or in many cases forbidden. The ability to employ non-invasive and non-destructive analytical methods that can be used in situ is essential in the study of such objects and opens new opportunities for the investigation of the materials these works are fashioned from.

This presentation will focus on Judd’s three-dimensional works made of metal and their finishes from 1964-1994. Portable instrumentation were utilized in this study, including handheld X-ray fluorescence spectroscopy (hhXRF), attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) and colorimetry. The advances in portable instrumentation have allowed for researchers to study a larger number of works - gaining a fuller understanding of the artist’s oeuvre - compared to the small number of actual samples that can be taken from the objects. Issues of the challenges and limitations of these portable techniques will also be discussed.

Keywords: Art/Archaeology, FTIR, Portable Instruments, X-ray Fluorescence
Application Code: Art/Archaeology
Methodology Code: Portable Instruments
Chemical Issues with Contemporary Art

Fluorimetric Analysis of the Constituent Dyes within Daylight Fluorescent Pigments: Implications for Display and Preservation of Daylight Fluorescent Artwork

Abstract Text

Daylight fluorescent pigments fade rapidly, accompanied by a chronology of colour change. Multiple organic fluorescent dyes are routinely incorporated into a given daylight fluorescent pigment, to either additively fluoresce or interact through energy transfer. The organic fluorescent dyes employed differ in photo-stability and upon loss of each species of fluorophore an abrupt colour change is observed. The collective result of this fading behaviour is that in a short period of time a daylight fluorescent paint layer will be of a different hue, devoid of luminosity. As consequence it is almost impossible to colour match a faded daylight fluorescent paint layer without the hues diverging asynchronously, or ascertain the original palette of a daylight fluorescent artwork after a protracted period of time. To ensure best practice in the preservation of artworks that contain daylight fluorescent paint here we perform a fluorimetric and chromatographic analysis of the DayGlo daylight fluorescent pigment range at the constituent dye level, prior to and during an accelerated light ageing program. This research finds that the complex dye formulations employed in daylight fluorescent pigment manufacture raise several implications for the display and treatment of this modern palette.

Keywords: Art/Archaeology, Chromatography, Fluorescence, Paint/Coatings
Application Code: Art/Archaeology
Methodology Code: Fluorescence/Luminescence
Miniature Mass Spectrometers

This talk will first review the motivation for mass spectrometer miniaturization, and then present results of our efforts to make small linear ion traps. I will present results on our sub-mm trapping dimension linear ion trap made using ceramic plates, and discuss current efforts and rationale for further miniaturization.

Keywords: Mass Spectrometry, Portable Instruments
Application Code: Environmental
Methodology Code: Mass Spectrometry
Miniature Mass Spectrometers
Where We Are in Mini MS and How We Got Here

This presentation surveys the history of small mass spectrometers from 1989 to the present. It emphasizes full, autonomous MS systems and covers analyzers, vacuum systems, and most importantly sample handing systems. Performance criteria of current instrumentation are summarized. Applications to fuels, cosmetics, pharmaceuticals, medicine, natural products, forensics and reaction monitoring are discussed. The combination of small systems like the Mini 12 system with paper spray, touch spray, DESI and other ambient ionization methods is of central importance.

Abstract Text

Keywords: Analysis, Mass Spectrometry
Application Code: Process Analytical Chemistry
Methodology Code: Mass Spectrometry
The past several decades have seen significant effort directed towards the development of compact mass spectrometry systems. However, realization of a mass spectrometer with a handheld form factor has remained elusive. Eliminating the turbomolecular pump typically utilized in transportable instruments significantly reduces the system size, weight, and power (SWAP). Submillimeter scale ion trapping structures have allowed the first demonstration of high-pressure mass spectrometry (HPMS), the generation of mass spectra at pressures exceeding 1 Torr, and a truly handheld mass spectrometry device that can operate for hours using its on-board batteries. Such pressures can be maintained at high gas throughput using small, rugged mechanical pumps, significantly reducing the instrument’s form factor and robustness. HPMS has led to the development of a handheld mass spectrometer that promises to be an order of magnitude smaller than currently available field deployable instruments. Moreover, the HPMS system utilizes ambient air as the buffer gas, essentially eliminating reagent logistics. The performance characteristics of these HMPS systems will be discussed.

Keywords: Mass Spectrometry
Application Code: Other
Methodology Code: Mass Spectrometry
The mass resolution of a TOF mass spectrometer is directly proportional to its total flight path length. Thus, the maximum flight path that can be achieved in a typical laboratory instrument is of the order of a few meters. In order to overcome this fundamental problem, it is necessary to store the ions in a closed orbit, and to pass the ions around the same orbit many times. However, if the ion beam diverges in both time and space, then both the mass resolution and the ion transmission are compromised as the number of cycles increases. To avoid this, we need to design an ion optical system that gives ‘perfect focusing’, where the ions return to their point of origin in the system, in both time and space. We found three ion optical systems that achieve the ‘perfect focusing’.

One of these proposed TOF systems, the ‘MULTUM Linear plus’, was constructed as a laboratory model for the ROSETTA space mission. It consists of four cylindrical electrostatic sectors and 28 electric quadrupole lenses. It was experimentally demonstrated that mass resolution increased according to the number of ion cycles through the ion optical system. A mass resolution of > 350000 was achieved after 500 cycles. Another multi-turn TOF mass spectrometer, the MULTUM II, was also developed in an effort to reduce the number of quadrupole lenses. The new instrument consists of only four toroidal electric sectors. MALDI ion source was attached to the MULTUM II for analysis of biomolecules. A mass resolution of > 100000 was achieved for Angiotensin I.

Recently, we are developing various types of mass spectrometer based on the MULTUM II technology, for example, a tandem TOF mass spectrometer ‘MULTUM -TOF/TOF’, a stigmatic type imaging mass spectrometer ‘MULTUM -IMG’, and portable mass spectrometers of high mass resolving power ‘MULTUM-S’ series.

I will talk about the outline of the multi-turn TOF mass spectrometer and the development history of the MULTUM-series and their applications.

Keywords: Imaging, Instrumentation, Mass Spectrometry, Time of Flight MS
Application Code: Environmental
Methodology Code: Mass Spectrometry
On the upcoming joint European Space Agency (ESA) – Roscosmos (Russia) ExoMars rover mission, the Mars Organic Molecule Analyzer (MOMA) investigation includes a linear ion trap MS under development with support from NASA. The goal of the ExoMars rover is to seek the signs of past or present life on Mars, and MOMA contributes to this by analyzing organic molecules that may be present in samples acquired by a two-meter deep drill. At such depths any complex organics may be well-protected from the punishing cosmic ray flux on Mars, which easily reaches the surface due to the lack of a global magnetic field. The MOMA investigation, led by Max Planck Institute for Solar System Research (MPS) in Germany, with PI Dr. Fred Goesmann, combines pyrolysis GCMS with Mars-ambient laser desorption MS (LDMS) modes to analyze powder samples from the drill.

The linear ion trap supports both electron ionization (EI) from the GC effluent, injected into the trap from one end of the hyperbolic electrode array; and laser desorption/ionization from the other end, with ions carried through a narrow tube from Mars ambient (5-8 Torr) to low pressure. The laser interface is a new development for space application, however it is based on the discontinuous atmospheric pressure ionization (DAPI) approach pioneered at Purdue University. A new check-ball type valve is used to close off the inlet tube after laser ions are trapped, permitting the analyzer pressure to reduce to below 1 mTorr where the high-voltage detectors can be activated. Another novel feature (and challenge) of the MOMA ion trap is that the residual Mars atmosphere, primarily CO2, is used as the bath gas in LDMS mode. A separate He tank and regulator is included in the University of Paris-provided GC, which supports pressurized flow and bath gas in the GCMS mode.

**Keywords:** Gas Chromatography/Mass Spectrometry, Ion Trap, Laser Desorption, Mass Spectrometry

**Application Code:** Other

**Methodology Code:** Mass Spectrometry
Sensors need good selectivity for accurate measurements on complex samples such as found in the environment. Spectroelectrochemistry offers a means of providing the selectivity to target a single analyte in a sample containing many potential interferences. In one type of spectroelectrochemical sensor the additional selectivity is provided by subjecting the analyte to electrolysis that electrochemically modulates an optical signal such as absorbance or fluorescence. The modulated signal can then be distinguished from the constant signals of the potential interferences, and the analyte quantified by the magnitude of the change in optical signal. This strategy can be implemented with an optically transparent electrode coated with a thin polymer film that preconcentrates the analyte for spectroelectrochemical detection. Spectroelectrochemical sensors using absorbance or fluorescence have been developed for detection of a variety of analytes: metal complexes; heavy metals such as lead, cadmium, copper and zinc; polycyclic aromatic hydrocarbons; and nitrate. Sensors have been demonstrated on complex samples such as radioactive waste from nuclear processing and natural water from the environment.

Keywords: Electrochemistry, Environmental/Water, Sensors, Spectroelectrochemistry
Application Code: Environmental
Methodology Code: Electrochemistry
Exposure to metals through ingestion of contaminated food and water is well established as a leading exposure route for toxic metals. Exposure through inhalation of particulate phase metals is poorly understood, largely because long sampling times (8-48 hrs) are needed to accumulate enough sample mass for analysis with traditional methods. Furthermore, traditional analytical techniques are expensive, creating a barrier to routine exposure assessment. Electrochemical Paper-Based Analytical Devices (ePADs) provide an alternative, low-cost tool for analysis of metals in particulate matter. ePADs can be both sensitive and selective for different metals when using either anodic or cathodic stripping voltammetry. Here, development of ePADs for analysis of Pb, Cd, and Zn using anodic stripping voltammetry (ASV) and Mn using cathodic stripping voltammetry (CSV) will be presented. Pb and Cd can be readily detected using screen-printed carbon electrodes with ASV. Zinc detection is poor at these electrodes, however, because of the large oxygen reduction background present at the same potential. Electrodes modified by electrospinning using a hybrid polyaniline/graphene film shift the reduction potential of oxygen more negative allowing for detection of Zn. Analysis of particulate samples collected in an industrial work environment were analyzed using this method. Next, analysis of Mn using CSV will be presented. Mn is present in many aerosols, and exposure elevated Mn in particulate matter has been linked with higher mortality rates. To create this system, carbon electrodes were screen-printed from a custom formulation onto thin polyethylene sheets. Benzoquinone was subsequently used as an internal standard and reduced device-to-device variability substantially. Atmospherically relevant detection limits have been achieved and aerosol samples have been characterized using the method.
Heavy metal analysis is typically made using techniques such as ICP-MS, where solution is taken from the source to the laboratory. However, there is now a move towards direct measurements at the source, with the capabilities for continuous monitoring. Electrochemistry is often touted as an ideal methodology with stripping voltammetry often the technique of choice where metals from solution are electroplated on the electrode (preconcentration) and then stripped from the surface to give a peaked analytical signal. The size of the signal and position of the peak on a potential axis is used to determine concentration and chemical identity. Since liquid mercury has been banned, solid electrodes must be used which complicate the measurement as multiple peaks can be returned for one metal,1 and alloying of metals on the surface results in peaks in positions which can lead to incorrect interpretation of solution composition. To overcome this problem we recently reported the development of a novel analytical technique, electrochemical X-Ray fluorescence (EC-XRF).2 In EC-XRF electrochemical preconcentration of a labile species of interest onto the electrode is achieved by electrodeposition. Unambiguous elemental identification and quantification of metal concentration is made using XRF, improving the limit of detection of energy dispersive XRF by over four orders of magnitude. Initial work focused on ex-situ measurements,2 however, work has now moved towards in-situ approaches where EC-XRF measurements are made directly in the fluid of interest. For trace metals which are not electrochemically labile, we also introduce a dual electrode configuration, where an outer or upstream electrode is used to generate known and controllable proton concentrations over the sensing electrode, freeing the complexed metal ion for subsequent electrochemical deposition or detection.


Keywords: Electrochemistry, Environmental Analysis, Environmental/Water, X-ray Fluorescence
Application Code: Environmental
Methodology Code: Electrochemistry
New Frontiers for Electrochemical Trace Metal Detection of Biological and Environmental Samples on Fast-Scan Cyclic Voltammetry (FSCV) for Rapid Environmental Analysis

Excessive levels of metals in urban surface waters are one of primary causes for water quality impairments. As a result, detecting, characterizing, and quantifying metals in these dynamic systems are critically important. Traditional techniques for measuring metals in aqueous samples are time consuming and expensive. In order to achieve sufficient sensitivity, most of the existing techniques used to monitor metals measure total concentrations and generally requires large sample volumes, which greatly reduces the spatial resolution and number of samples it is logistically feasible to collect.

Unfortunately, most metals undergo extensive aqueous complexation and have multiple redox states, both of which control the fate and transport as well as the toxicity of metals. We have recently applied Fast-Scan Cyclic Voltammetry (FSCV) to assess the behavior of metals in the environment. To demonstrate the utility of this novel analytical technique we will show how it was used to assess the complexation of copper with dissolved organic matter (DOM) and the effect of competitive aluminum binding on copper speciation. In another example, we will show how this small electrochemical sensor (2 mm diameter) can be used [i]in vivo[/i] to characterize the speciation and transport of arsenic. Finally, we will present emerging applications for this novel electrochemical technique that will help to address critical knowledge gaps in environmental science and engineering.

Keywords: Electrochemistry, Environmental, Environmental Analysis, Geochemistry

Application Code: Environmental
Methodology Code: Electrochemistry
Trace metal pollution is a critical environmental issue to address. Natural and anthropogenic sources of trace metals can be toxic to plants, animals, and humans. There is therefore great interest in reducing their impact. Trace metals often exert their most polluting effects during hydrodaulic events (storms), which occur dynamically. To efficiently implement metal mitigation systems, more information is needed about dynamic, aquatic trace metal chemistry. This kind of information can be provided by an in-situ analytical device that is portable, low-cost, low-energy, and submersible while providing a continuous measurement output. Electrochemistry has shown promise for this goal in the past but it has been limited by its temporal resolution and concerns about Hg toxicity. We have recently shown that fast scan cyclic voltammetry (FSCV) at carbon fiber microelectrodes is an ideal analytical tool for real-time trace metal detection. In this work, we describe the fundamental surface mechanisms of FSCV for Cu(II) analysis. We study surface adsorption mechanisms in a variety of ways and derive the thermodynamic adsorption relationships between the carbon fiber surface and solution ligands. Finally, we discuss novel methods for modifying carbon fiber surfaces for increased selectivity when analyzing authentic stormwater samples.

**Keywords:** Chemically Modified Electrodes, Environmental/Water, Microelectrode, Surface Analysis

**Application Code:** Environmental

**Methodology Code:** Electrochemistry
Thermal analysis is the measurement of some physical property of a material as a function of temperature. The properties of interest include heat capacity, heats of transition, mass loss, expansion/contraction, modulus and many others. While not all properties may be measured over all temperatures, the application range of commercial apparatus is -170 to 1600 °C. A brief history of commercial thermal analysis instrumentation is provided along with rationale for development and trends. The Kondratiev Cycle is used to model the growth of the industry to its present maturity along with the Boston Consultation Group model for its present and future. Leading contributions to the art of Robert Stone (Stone Company), Jen Chiu (DuPont Company), Mike O’Neill (Perkin Elmer), Dick Baxter (DuPont Company), Mike Reading (ICI) and others will be recognized.
The first thermal analysis measurements were made during the 19th century when cooling curves were used to investigate phase transitions. Roberts-Austen created the first differential thermal analysis instrument by adding a second thermocouple to measure the difference in temperature between the sample and an inert reference. This increased the sensitivity of the apparatus. The temperature difference measurement combined with a sample temperature measurement remains the basis for all modern differential thermal analysis and DSC. Vold applied a heat transfer analysis to her differential thermal analysis instrument to obtain sample heats of transformation, thus adding quantitative heat flow rate measurements, i.e. scanning calorimetry to differential thermal analysis. The lumped heat capacity formulation she employed continues to be the basis for obtaining heat flow information from DSC. Boersma described a differential thermal analysis instrument having high thermal conductivity sample and reference holders configured such that the differential temperature measurement was largely independent of the sample thermal conductivity, improving the reproducibility of the differential temperature measurement and hence increased precision of heats of transition. The early 1960s saw the introduction of the first commercial DSCs and the name differential scanning calorimeter was first used. Power compensation DSC was introduced along with heat flow DSC derived directly from differential thermal analysis. Temperature modulated DSC developed by Reading added periodic temperature oscillations to temperature scanning, allowing the resulting heat flow signal to be separated into a heat flow due to the sample heat capacity and a heat flow due to latent effects such as crystallization. Rapid heating and chip DSC have recently been developed that expand heating and cooling rates up to 105 K/min and beyond to facilitate analysis of metastable materials.

Keywords: Instrumentation, Temperature, Thermal Analysis
Application Code: Materials Science
Methodology Code: Thermal Analysis
Hyphenated or coupled techniques have been of interest in thermal analysis for decades. Applications of evolved gas analysis (EGA) techniques were a research topic in the late 1980s and early 1990s as analysts realized that techniques like thermogravimetric analysis were much more powerful when the off-gases were precisely identified. Other techniques like spectral DSC or DMA developed as the need for more precise understanding of the thermal transitions or thermo-mechanical changes in a material. Starting with a historical review, we will look at the development and current state of hyphenated techniques including EGA, spectral-thermal, and sample modification methods.

Keywords: FTIR, GC-MS, Mass Spectrometry, Thermal Analysis
Application Code: Other
Methodology Code: Thermal Analysis
Thermomechanical Analysis (TMA) is basically measuring physical dimensions as a function of temperature and force. Although the idea that materials generally expand when heated has been well known, it was not until 1948 that the relationship between dimensional changes with temperature were quantified and a technique standardized. This was also when the term “Thermomechanical Analysis” came into use. The first commercial TMA instrument became available in the 1960’s. In the 1980’s with the advent of Linear Variable Differential Transformers, the precision of TMA instruments greatly improved. Current TMA instruments can measure dimensional changes with a variety of fixtures in compression, penetration, tension, or flexural modes with zero applied force (contact), constant force (stress), or constant strain. In addition to linear dimensional changes, volumetric changes can be measured with special containers. Although TMA basically measures thermal expansion which usually varies linearly over short ranges, the applications are very diverse.
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**Abstract Text**

Over the past 20 years, bisphenol A (BPA) has come to define an entire class of risks to the public from consumer products thanks to extensive media coverage. As such, it has also come to symbolize a broken regulatory system, one that is incapable of responding to new analytic techniques in toxicology and new understandings of endocrinology. Yet a close analysis of the way the media has covered BPA within the context of endocrine disruption and the broad notion chemical risk reveals systemic unbalanced coverage, narrative bias, and a chronic lack of methodological and statistical understanding. The result is that the public has been encouraged to think of BPA as representing the quintessence of chemical risk while regulators have become ever more assured of its safety. How did this happen, and what-if anything-can be done about it?

**Keywords:** Beverage, Chemical, Environmental/Water, Food Safety

**Application Code:** Other

**Methodology Code:** Other
Although numerous food regulatory bodies continue to support the safety of bisphenol A (BPA) as a food contact substance, consumers remain wary of products containing BPA. To satisfy consumer demand, can manufacturers and food producers have been developing alternative can coatings to replace epoxy coatings based on BPA. The current FDA Guidance for food contact substances recommends that if the product is retort pasteurized or sterilized, a retort step (121 [degree]C for 2 h) should be performed, followed by a migration test during 10 days at 40 [degree]C. Traditionally, 10 day migration tests at 40 [degree]C have been consider appropriate to simulate migration from packaging into food. However, can coatings can be stored in contact with food for years, and there is very little validated migration data evaluating migration for extended time periods. The scope of this study is to evaluate if the procedures recommended by FDA guidance are appropriate to accurately assess migration for long term storage. To carry out the experiment, four types of can coatings including: Polyester, acrylic-phenolic, epoxy-resins and vinyl coatings were used. After identification of potential migrants, a final set of compounds including monomers and related substances belonging to the coatings were used for tracking migration. Migration times were from 1 day to 1.5 years at 40 [degree]C. Concentrations of the migrants were determined in the different food simulants. For some of the compounds initial data shows an increase in migrant concentration beyond the traditional 10 day time point. These results suggest that migration protocols may need to be modified to address long term storage.

Keywords: Characterization, Chromatography, Food Science, Polymers & Plastics
Application Code: Food Safety
Methodology Code: Sampling and Sample Preparation
The quality of food can be positively, and sometimes negatively, affected by its interactions with packaging materials and their environment. Interactions such as migration and sorption with inks, laminates, over-lacquers or seal coatings are often causes for off aromas and flavors in foods. Furthermore, migration can also have positive effects on the quality and safety of food. Use of antioxidants or antimicrobial agents can improve shelf life and microbial safety of products when incorporated into the packaging material. A brief summary of incidents of migration and sorption having negative effects on food will be covered as well as important regulatory considerations. Lastly, development of packaging materials having an intended positive effect will be summarized. This will mainly focus on antimicrobial food packaging research involving incorporation of nisin for reducing microbial growth in ready-to-eat meats.

Keywords: Food Safety, Food Science
Application Code: Food Safety
Methodology Code: Other
Recent technological developments in active food packaging enable exciting new applications. In this work we describe synthesis of a non-migratory active packaging material prepared by photoinitiated surface graft polymerization which is capable of chelating metal ions from packaged food products. Surface analysis of materials was performed using Fourier transform infrared spectroscopy, inductively coupled plasma mass spectrometry, colorimetry, and electron microscopy. Attenuated total reflectance Fourier transform infrared spectroscopy revealed absorbances characteristic of the desired chemistries at each stage of the modification, including grafting of photoinitiator, methacrylic acid monomer, and converted hydroxamic acid moieties (see figure). Polypropylene, polyethylene, and polyethylene terephthalate were successfully surface modified to possess chelating moieties including acrylic acid and hydroxamic acid, grafted via covalent linkages. Utilization of the hydroxamic acid chelating moiety improved retention of chelating properties at pH values as low as 3.0. Our chelating active packaging materials were shown to have wide application, including inhibiting lipid oxidation in emulsions and improving activity of natural antimicrobials such as lysozyme. This work demonstrates the potential for using active food packaging as a means to reduce food additive use, specifically use of metal chelators such as ethylenediamine tetraacetic acid.
Migration is an important determinant of the potential consumer exposure to nano-components in food contact materials (FCMs). Therefore, characterizing the physico-chemical properties and potential for migration of constituents is an important step in assessing the safety of FCMs. An evaluation of a number of commercially available nano-enabled FCMs purchased domestically within the US and internationally using the current FDA Guidance for evaluating migration, showed the detection of sub–ppb levels of silver in food simulants. The migration trend was characterized by a surface desorption phenomenon, and nanoparticle migration was not observed. Based on the above observations it is important to evaluate the potential for nanoparticle release under conditions that significantly change the exposed surface area of the FCMs. Data from migration analysis performed under stressful use conditions will be presented.

**Keywords:** Electron Spectroscopy, ICP-MS, Nanotechnology, Polymers & Plastics

**Application Code:** Food Safety

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
At temperatures above approximately 7ºC, microbial spoilage of some perishable foods can begin within hours. In order to mitigate spoilage during the storage, transportation and handling of perishable foods, use of antimicrobial packaging can be very effective. Ethyl pyruvate is an effective antimicrobial and classified as GRAS (generally recognized as safe) by the U.S. Food and Drug Administration for common use in food applications. In this study, GC/MS was used to evaluate the effective permeability of four film structures for gas phase diffusion of ethyl pyruvate (EP) into the headspace of a food storage bag. The information gained provided insight toward optimized design of a laminate food storage bag which includes unperforated outer and inner layers and an intermediate layer which includes a solventless adhesive mixed with EP. The objective of the study was to determine an effective inner film layer.

Five food packaging films were evaluated: Linear Low Density Polyethylenes (LLDPE, 0.80mil (control) and 1.00mil); Ethylene Vinyl Acetate (EVA, 1.00mil); Low Density Polyethylene (LDPE, 1.00mil); and Metallocene Polyethylene (MPE, 1.25mil). Headspace vials containing microvials with 5uL EP and sealed with the sample films were sampled every 3-4 hours using static and SPME HS. Analysis was performed on an Agilent 6890 GC with DB5-MS column coupled to an Agilent 5973 MSD. The results for static HS are shown in Figure 1. The results show that MPE has a significantly higher permeability than the other films tested, and may be a good candidate for packaging inner layer structure in EP antimicrobial laminate packaging.
High Performance Redefined for the Clinical Laboratory: New Technologies and Methods to Enable Per

Applications for the Quantification of Proteins from Dried Blood Spots (DBS)

The past decade has seen significant investigation into expanding the use of Dried Blood Spots (DBS) beyond neonatal screening. Most of this research has focused on nucleic acids or small molecule applications. Recently, investigators have reported success with the quantification of proteins from DBS. The work presented by SISCAPA assay technologies is of particular interest to OpAns. Leveraging our current work with the quantification of small molecules in clinical DBS samples and protein/peptide quantification using pull down enrichment, we have investigated the quantification of proteins from DBS using the SISCAPA approach. We will discuss challenges with the methodology, solutions that we found, and the practical applications of the technique in our hands.

Keywords: Clinical/Toxicology, Liquid Chromatography/Mass Spectroscopy, Protein, Small Samples
Application Code: Clinical/Toxicology
Methodology Code: Liquid Chromatography/Mass Spectrometry
Serum thyroglobulin is measured as follow up assessment for treatment of thyroid cancer. Traditionally, serum thyroglobulin has been measured by immunoassay, which have excellent sensitivity (0.01 ng/mL). However, several studies have pointed out the presences of antibodies to thyroglobulin in about 10% of the population, which cause interferences in an immunoassay. The percentage is even higher in patients with differentiated thyroid carcinoma. Mass spectrometry is a growing tool for clinical diagnostics especially in the quantitation of small molecules, but has had less growth in the quantitation of proteins. The importance of accurately measuring thyroglobulin concentrations in serum and the problems with immunoassays has made it necessary to develop mass spectrometry techniques to solve this problems. This talk will discuss the current status of assays and patents for thyroglobulin quantitation.
High Performance Redefined for the Clinical Laboratory: New Technologies and Methods to Enable Per
Extraction, Preparation and Quantitative Analysis of Protein Biomarkers in Biological Fluids

Today's clinical laboratories offer many tests that can be used to detect a number of diseases. In some cases, the
quantitative analysis of small-molecule biomarkers in biological fluids can be indicative of a disease state, though it is
commonly believed that proteins, especially low abundance proteins and their modifications, are a rich source of
biomarkers with greater potential for the detection of disease than small molecules. This paper will examine the current
limitations of traditionally employed sample preparation and analysis techniques for protein biomarkers in biological
fluids. A novel platform for the extraction and quantitative analysis of protein biomarkers will be discussed, using several
model compounds for illustration.

Keywords: Bioanalytical, Biological Samples, Clinical Chemistry, Liquid Chromatography/Mass Spectroscopy
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Objective and Significance
Micro flow LC-MS/MS (MFLC-MS/MS) has been shown to deliver significant sensitivity gains over traditional HPLC-MS/MS, while providing additional benefits such as lower required sample volumes and solvent consumption. At these µL/minute flow rates, the ESI-MS source can be coupled with the MFLC column ("the column is inside the source") to further improve MS signal and chromatography compared to traditional segregated techniques.

Procedures and Equipment
Organic precipitation was used to prepare samples of human methotrexate (MTX) in plasma for analysis by MFLC-MS/MS. An Eksigent Express HT-Ultra LC® system coupled with an ABSCIEX 6500® QTRAP mass spectrometer was used for MFLC-MS/MS analysis.

Results
Accuracy and precision for the analysis of MTX from human plasma was better than ±13% at all levels of QC samples (LLOQ to ULOQ). A stable-labeled internal standard was utilized for quantitation. Sensitivity, selectivity, accuracy, and precision were acceptable for routine bioanalysis and compared very well to conventional HPLC-MS/MS results.

Conclusion
Here we have demonstrated a validated, high throughput method for bioanalysis using ESI-MS/MS coupled with the MFLC column. This data, along with the sensitivity and efficiency benefits of MFLC-MS/MS, suggests that the technique has value as a technique in the bioanalytical or clinical laboratory.

Keywords: Liquid Chromatography/Mass Spectroscopy
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Mass spectrometry (MS) is an efficient, sensitive, and highly specific detector for a wide range of compounds encountered in the clinical laboratory. The addition of tandem mass spectrometry enables targeted quantitation of both small and large molecule of endogenous and exogenous origin. The sample preparation and introduction method of choice for mass spectrometry has been reverse phase liquid chromatography (RPLC). The peak capacity and resolution of RPLC has been an essential part of the success of MS in the clinical laboratory.

Unfortunately the greatest strength of LC for LC-MS is also its greatest weakness. LC is an inherently analog technology. Considerable effort in method development and technical overhead (operator expertise) is required for success. Much of the down time spent in high throughput laboratories is split between method development followed by downstream support of the LC instrumentation. High quality chemical separations take time, both to develop the method, as well as to achieve each individual separation per sample. Chemical separations occur on the minutes to hours time scale.

In contrast MS is inherently digital. Ions are generated and analyzed at a rapid rate in discrete packets. MS is acquired on the microsecond to millisecond time scale. The LC in LC-MS is limiting the adoption of MS in the clinical environment.

We present an approach to digitize the LC-MS workflow to alleviate these limitations. By segmentation of the LC sample stream into 1- or 2-dimensional microfluidic arrays, the chromatogram may be moved from an analog environment to a digital workflow. This simple paradigm shift fully decouples the mass spectrometer from the LC. Since the MS is no longer tied to the timescale of LC, it can be applied in totally new ways that would not be economically feasible by traditional LC. Some initial results and possible directions for this new technology platform will be presented in the analytical laboratory.
Gaining Insight into Complex Biology Using Targeted Quantitative μLC-MS

Quantifying the ability of a dosed therapeutic to disrupt the interaction between an endogenous protein ligand and its receptor is a complex biological question. To fully address this complexity, the Discovery Bioanalyst must often provide an understanding of the preclinical disease model, demonstrate on-target effect, and accurately quantify target load in the diseased tissue. Complete PK/PD modeling is then possible considering perturbations of biomarkers, ligand, and receptor quantified in both circulation and resident to tissue. Bioanalytical assays to provide this data require exquisite sensitivity due not only to extremely low endogenous levels, but also limited sample amount. Additionally, multiple measurements are often made from a single sample in order to describe various protein-protein interactions proximal to drug action to provide strong proof-of-mechanism. To accomplish these measurements, we incorporate sample preparation using immunoenrichment followed by low flow LC-MS technology. By intelligently designing assays to capture specific protein populations of interest, coupled with selective and sensitive detection of multiple target proteins, we are able to provide critical PD information. In this presentation we highlight key LC-MS technologies and sample preparation approaches that have enabled an unprecedented depth of understanding during the development of biotherapeutics. These understandings could ultimately impact personalized healthcare, providing guidance to patient selection and proper dosing.

Keywords: Bioanalytical, Capillary LC, Drug Discovery, Liquid Chromatography/Mass Spectroscopy
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
In the manufacturing of pharmaceutical drug substances, it may be necessary to demonstrate that impurities are below an appropriate safety threshold. The threshold is defined by the International Conference of Harmonization (ICH) and determined by the target dose and route of administration. Robust methods are needed to perform impurities testing on the final drug substance as well as starting material, intermediates, and critical control points in a synthesis. This presentation will review case studies involving method development of trace-level impurities in drug substances as well as starting materials and intermediate. Analytical techniques, such as chemical derivatization, HPLC, and MS, which are used in developing methods for the control and monitoring of impurities and degradation products will be discussed.

Keywords: Derivatization, HPLC, Mass Spectrometry, Pharmaceutical
Application Code: Process Analytical Chemistry
Methodology Code: Liquid Chromatography/Mass Spectrometry
A pharmaceutical impurity is any component that is not the chemical entity defined as the drug substance or an excipient in the drug product. The drug impurity can be organic or inorganic impurity, residual solvent and filter aids. The organic impurity may result from the starting materials, process-related impurities, intermediates, API and degradation products. Due to the apparent impact of impurities on pharmaceutical quality, the identification of any impurity >0.1% area in relationship to the API are generally required. The common impurity isolation and identification technologies include LC/MS, LC/HRMS, HPLC, SFC, prep-HPLC and prep-SFC, 1D/2D NMR, re-crystallization and sample degradation. Isolation of low level drug impurity (~0.1-1%) can be very challenge. The deep understanding on the isolation technologies, chromatography, and the theories on the impurity formation/pathways are critical for the success. In this presentation, we will provide a general overview for impurity isolation strategy including various purification technologies, column chromatography, analytical method development for optimizing the resolution as well as the discussions on impurity structure work flow.

**Keywords:** HPLC, SFC
**Application Code:** Pharmaceutical
**Methodology Code:** Liquid Chromatography
As a drug candidate moves successfully through the development process it becomes increasingly important to identify drug related impurities. Isolation of low-level (0.1-1.0%) drug product degradants for structure identification can prove very cumbersome and time consuming when going directly through the drug product as a sample source. Low level impurities in low dose formulations with limited sample supply will significantly limit the amount of impurity material one can recover. Sample enrichment becomes a critical step to support the structure elucidation process. One can exploit the degradation chemistry “toolbox” by generating targeted screens with a narrow approach in experimental design. Case studies will be presented to highlight sample enrichment and isolation process that ultimately support MS and NMR structure elucidation of targeted low level drug product degradants.

Keywords: Identification, Prep Chromatography, Separation Sciences, Supercritical Fluid Chromatography

Application Code: Pharmaceutical

Methodology Code: Other
In many cases, routine 1D NMR methods are sufficient for structure elucidation of relatively simple low molecular weight compounds. However, as the complexity of molecular structure increases, interpretation of 1D NMR spectra becomes challenging. Use of more powerful homonuclear and heteronuclear 2D correlation NMR spectroscopy addresses this challenge for small to medium size molecules. Practical strategies and tactics for establishing structural connectivity using 2D NMR methods will be discussed. Case studies of the structure elucidation of impurities in drug substances will be presented.
### Abstract Text

Development of new chemical entities (NCE) with high chemical and chiral purity is a regulatory expectation in new drug development. Today, it is not unusual to have complex molecules with 3 or 4 stereogenic centers which show high affinity for disease targets. For the process development of these “multi-chiral” molecules, numerous analytical methods capable of separating all stereoisomers (enantiomers and diastereomers) must be developed rapidly to assess and control the stereochemistry of raw materials, intermediates and the final active pharmaceutical ingredient (API). Achiral reversed-phase (RP) methods, used to assess the overall chemical purity assays, can typically be developed to monitor the diastereomeric content in a single run. In many cases, these achiral methods often become the primary quality control (QC) and stability-indicating purity assay methods. This paper describes the method development and QC strategies used for these complex multi-chiral drug molecules, which include:

- Adoption of a 3-pronged HPLC method development template approach
- Development of a single RP method using multi-segment gradients for determination of overall purity and diastereomeric content
- SFC for rapid initial screening for chiral separations
- 2-D LC for peak purity determination and achiral/chiral combinational assays

These strategies will be described with actual examples used during clinical development of several new drug candidates.

### Keywords

- Liquid Chromatography
- Pharmaceutical
- Quality
- SFC

### Application Code

- Pharmaceutical

### Methodology Code

- Liquid Chromatography
Chromatographic isolation of impurities and minor components in complex mixtures can be extremely challenging. Often times the impurity or minor component to be isolated is structurally very similar to the parent component, making it necessary to perform chromatographic method development in order to establish pure analyte. The use of the isolated trace level compounds range from full characterization via MS-MS and NMR to evaluation of biological activity and toxicity making quantity isolated as important as purity. This presentation will detail the modes of chromatography used for these separations and will highlight modifications made to preparative instrumentation traditionally used in the high-throughput arena to maximize performance for these challenging samples. Characterization data generated for the isolated compounds will be presented to demonstrate the array of information that can be derived from the small sample size with state of the art instrumentation.

Keywords: HPLC, Isolation/Purification, Liquid Chromatography/Mass Spectrosocpy
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
The chromatographic separation and the analysis of polar compounds using HPLC has always been a challenging. It would seem that HPLC analysis would be well suited for the determination of the polar compounds however routine reverse phase HPLC analysis has yielded poor quality results for these types of compounds. The analysis of polar compounds via routine HPLC analysis has been deficient in several main aspects including poor retention, unacceptable low k' values and poor peak shapes. One of few HPLC techniques capable of analyzing polar compounds has been traditional normal phase chromatography (polar stationary phase with a non-polar mobile phase). Unfortunately, normal phase chromatography suffers from several major deficiencies including poor retention time reproducibility, poor column to column reproducibility and very low or no solubility of the target analytes. A number of HPLC stationary phases have introduced to improve the analysis of polar compounds. These HPLC stationary phases include high aqueous stable phases, HILIC (hydrophilic interaction chromatography) phases and polar embedded phases all of which utilize various levels of water in the mobile phase. It is the focus of this presentation to compare and contrast the HPLC modes developed for the analysis of polar compounds with a particular focus on HILIC mode. As part of our presentation we will introduce new HILIC stationary phases optimized for the separation of polar compounds. The goal of this work is to present a strategy for the HPLC analysis of polar compounds using HILIC conditions. In addition, the potential limitations of HILIC chromatography will be discussed including dynamic/linear range, retention time reproducibility, mobile phase composition and potential sample matrix effects.
Reflections on a 39 year career in the petrochemical industry during the time when women were just entering the spectroscopy profession. I will describe some career choices, industrial practices, and experiences of women in my lab during this time. The question of "Must Women Try Harder" is still relevant today - especially with concerns about advancement opportunities, "leaning-in", and self-confidence issues.

Keywords: Infrared and Raman, Petrochemical, Spectroscopy, Teaching/Education
Application Code: General Interest
Methodology Code: Other
In this talk I will describe how I ended up at HORIBA and how my career evolved the way it did. My academic work was in solid state physics at the University of Pennsylvania, and from there I moved to the Biophysics Department in the Medical School where I built a Resonance Raman instrument for studying interactions between hemes in functioning mitochondrial membranes. Because I was unable to secure a stable position in that department I accepted a position with ISA (also known as Instruments SA, and then later known as Jobin Yvon and finally HORIBA Scientific) to serve as the interface between potential users of Raman microscopes and the sales group. At the time (1978) the field was wide open, so product acceptance required extensive collaborative efforts to demonstrate the possibilities of the equipment. It was gratifying knowing that some of the measurements that I was making were being done for the first time, certainly in North America, and often in the world. And the best part was that the people who had requested the measurements were almost without exception appreciative, and invited me to publish with them. It is also worth commenting that for sure a large part of my success was due to the "corporate culture" that existed, and continues to exist in the company, which encouraged technical excellence and interpersonal cooperation.
This talk will reflect upon the career path taken thus far (25+ years) by one women chemist. Many steps - from graduate school to full professor - required taking chances and trusting in one's instincts. Yet until reflecting upon this path, the significant risk taking and serendipity were not necessarily apparent. And the challenges and benefits could not have been predicted at the outset.
None of us were able to get to where we are today without a little help! One way to increase the number of women who pursue science involves actively creating an environment that not only encourages but also supports women. This environment does not have to be created by women alone! Everyone can help in their own way to create such an environment to help inspire more young women to pursue science.

Each of us can encourage women in science through demo events, allowing students to shadow you for a day, or talking to a local class, showing students what science looks like is a valuable part of inspiring women to pursue science. Highlighting historic and current female scientists can also provide concrete examples of role models. When women see other women with successful science careers, they are more likely to believe that they too can have a successful science career.

An environment that supports women in science involves several components including emotional, verbal, and institutional support. Women are presented with many challenges that do not affect men in the same way, and providing an open ear or access to counselors can help women feel that they are accepted as part of the community. Verbally supporting women to show that women and men are valued equally, is one of the easiest things we can all do, but often forget. Finally, institutional support such as compliance with Title IX, childbirth accommodations, and leave options for both fathers and mothers not only support women, but also help more women continue their careers after having children.

Creating an environment that encourages and supports women in science is not easy, but we can all make small changes in what we do and say to work towards more equality. It’s also important to remember that these changes do not have to come from people who are already settled in their careers, young women can help too!

Keywords: Education, Teaching/Education
Application Code: Other
Methodology Code: Education/Teaching
Career choices in the sciences are often presented as black and white, like do you want to work in a lab or teach in academics or go to medical school? Starting into the workforce teaching high school chemistry and physics, with a goal of teaching at the university level, the analytical instrument business was never on the radar. Yet, the last 15 years in the instrument industry have been an incredibly fun and stimulating journey through roles as product manager, product specialist, and direct sales. Each transition brought growth and an enhanced personal skill set, however, one passion has woven itself through every facet of this exciting ride: an uncontainable love of teaching. Teaching chemistry and physics at the high school level, TAing chemistry in graduate school, and analytical and instrumental courses in a university setting offered traditional and enjoyable years in teaching, but the best was yet to come. This presentation will share personal experiences throughout three decades in analytical chemistry in both academics and the instrument industry with an emphasis on the more unconventional teaching roles, opportunities, and unique skills development that have served as career highlights in the last 15 years in the corporate arena.

Keywords: Fluorescence, Raman, Spectroscopy, Teaching/Education
Application Code: General Interest
Methodology Code: Education/Teaching
Madame Marie Curie served as my inspiration to become a woman scientist, and along the way I became a spectroscopist. My start began in physics like Marie Curie but evolved into physical chemistry and then a love for spectroscopy that has concluded at the National Institutes of Health in a study of biomedical solutions. To me, spectroscopy is the key to unlocking the many puzzles of science, all disciplines of science. With a physical chemistry degree in spectroscopy, I began my career in a radiochemistry lab working in food science and then quickly moved into spectroscopy of pesticide chemistry and instrument development for metabolites, then into toxicology which evolved into forensic toxicology and spectroscopic research in forensics. After the attacks of 9/11/2001 and the anthrax letters that followed, a lot of government work turned its focus to detection of biologicals that might be used as weapons. This evolved into the research of instrumental development for disease diagnosis and finally to the current position that I hold as Scientific Review Officer at the National Institutes of Health for the Instrumentation and Systems Development study section. In all roles, spectroscopy has played a major part in finding solutions for every different discipline that crossed my path. Along the way, I married (a Spectroscopist), had children (one who also became a woman scientist), led two national Professional Organizations as their elected President, and enjoyed a family life (hobbies will wait for retirement). Women in Spectroscopy can be successful and be women too.

**Keywords:** Bioanalytical, Biomedical, Biospectroscopy, Molecular Spectroscopy

**Application Code:** Bioanalytical

**Methodology Code:** Biospectroscopy
Women in Spectroscopy

A Career in Spectroscopy Publishing - 27 Years and Counting!

I will discuss some of my experiences, during a career that has spanned over 27 years, of commissioning and publishing in varied formats across the many different techniques of analytical chemistry. I will illustrate how publishing has evolved during this time from print books, journals and magazines, through licensed on-line content and functional e-books, and how these have reflected the evolution of spectroscopic techniques over this period, with some humorous anecdotes about my authors (no names!) and colleagues.

Keywords: Analysis, Instrumentation, Quality Control, Spectroscopy
Application Code: Other
Methodology Code: Education/Teaching
### Women in Spectroscopy: Technical Credibility and Excellence

**Abstract Title**

Technical Credibility and Excellence

**Primary Author**

Diane G. Schmidt  
University of Cincinnati

**Abstract Text**

Challenges and opportunities to achieving technical credibility and excellence will be presented.

**Keywords:** Education  
**Application Code:** Other  
**Methodology Code:** Education/Teaching
The d-forms of multiple free amino acids have been found in the central nervous and endocrine systems of both vertebrates and invertebrates. While progress has been made in understanding the significance of some d-amino acids (D-AA) such as D-serine (D-Ser) and D-aspartate (D-Asp), there remains interest in the characterization of less common d-amino acids. Progress has been complicated by the heterogeneous nature of these tissues, low endogenous concentrations, as well as sample volume constraints. Capillary electrophoresis coupled with laser-induced fluorescence (CE-LIF) is well suited for the analysis of low concentration analytes when working with small sample volumes, such as single cell samples. In this work, levels of the well characterized D-Asp and the less understood D-glutamate (D-Glu) in single sensory neurons from Aplysia californica are measured via chiral CE-LIF. A. californica nervous system was selected as it is an established neurobiological model, where the relationships between the activity of specific neurons and behavior have been linked. The approach used here involves sample pretreatment via methanol extraction, sample reconstitution and derivitization with naphthalene-2,3-dicarboxaldehyde for the measurement of the D-AAs. Additionally, the CE electrolyte included quaternary-ammonium-cyclodextrin for chiral resolution as well as employed large-volume sample stacking with an electroosmotic flow pump for sensitivity enhancement. The ability to separate and measure d-amino acids from single neurons appears well-suited for aiding in their characterization in the CNS. This work was funded via NSF CHE-1111705.
Oxygen Response to Local Application of Glutamate in the Nucleus Accumbens

Functional hyperemia is a biological mechanism of maintaining homeostasis in the brain through mediating regional cerebral blood flow (CBF) responses to regional neuronal activity. As a neuron fires, CBF increases provide blood rich with oxygen and glucose to renew locally depleted energy sources. Decoupling this system can result in Alzheimer’s or other disease states. Neurotransmitter dysregulation is also prevalent in neuropathies, making the relationship between CBF and neurotransmission important to understand. Excitatory glutamate neurons make up the largest percentage of neurons in the brain, but their behavior is best characterized in the cortex or in slices. We aim to better understand glutamatergic neurovascular influence deeper within an intact brain.

This research explores cell firing and oxygen responses to localized administration of glutamate to make inferences about its role in regulating vascular responses in the nucleus accumbens. Studies linking glutamatergic signaling to drug addiction behavior in the mesolimbic pathway has given more attention to this neurotransmitter in the nucleus accumbens. Measuring oxygen changes following glutamate-elicited neuronal activation serves as an indirect measure of blood flow changes to the area, and the neuronal activation can be measured using electrophysiology. Iontophoresis is used to locally deliver glutamate and drugs in close proximity to a neuron, and local oxygen is monitored using fast-scan cyclic voltammetry. Glutamate and oxygen changes, as well as neuron firing can be investigated using electrochemistry at a single probe. We present the results of iontophoretic application of glutamate and receptor-specific antagonists to cells in the nucleus accumbens.

Keywords: Electrochemistry, Microelectrode, Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Glutathione (GSH) serves as a reductant to maintain an appropriate redox status in cells. Abundant evidence shows that cells can be damaged during oxygen-glucose deprivation followed by reperfusion (OGD/RP). We wish to understand the effects of OGD/RP on neurons and astrocytes and study the intracellular redox status change in term of GSH response.

We have developed an approach using the organotypic hippocampal slice culture (OHSC) and green fluorescent protein (GFP)-based sensors [1] which are selectively sensitive to GSH redox state changes. The GFP probe, excited at 405 and 488 nm, responds in seconds to GSH redox changes (GSH as reduced form, GSSG as oxidized form). The quantity \([\text{GSH}^2]/[\text{GSSG}]\) (Nernst) can be determined from the fluorescence emission ratio.

Our study focuses on the GSH redox changes in mitochondria and cytoplasm of pyramidal cells and astrocytes in OHSCs. We treated OHSCs with 20 min OGD / 30 min RP, followed by fully oxidizing and reducing them for calibration. We find significant changes in the quantity \([\text{GSH}^2]/[\text{GSSG}]\) in mitochondria during OGD-reperfusion, but not in cytoplasm. This is the first time that \([\text{GSH}^2]/[\text{GSSG}]\) has been monitored in real time in tissue non-invasively. Supported by NIH Grant R01 GM066018.


Keywords: Biosensors, Fluorescence, Microscopy, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Fluorescence/Luminescence
Exercise invokes changes in the brain reward pathway similar to those observed with drug use. We are investigating how signaling peptides in the hippocampus of male C57BL/6J mice change with exercise using two mass spectrometry-based quantitation methods. The quantitative approaches are label-free matrix assisted laser desorption/ionization (MALDI) MS and stable isotopic labeling with LC-electrospray ionization (ESI) MS. To create our behavioral groups, half of the animals had access to an exercise wheel for 30 days while the other half remained sedentary. Tissue samples were isolated from the hippocampus of each animal. For MALDI MS analysis, an internal standard was added to tissue extracts prior to analysis. Profiles were collected for individual animals and peak heights were normalized to the internal standard. Seventeen candidate peaks were found to statistically differ between the two behavioral groups. For relative quantitation using isotopic labeling, extracts were pooled in groups of three and labeled with H[sub]4[/sub]- or D[sub]4[/sub]-succinic anhydride. Differentially labeled samples were combined and analyzed by LC-ESI-MS to obtain ratios of peaks relating to relative abundance in the two samples. The chromatographic separation of the sample leads to an increase in the observed peptides and so additional candidate peaks have been observed. The two quantitation methods provide complementary measurements and of the seventeen candidate peaks from MALDI MS analysis, six show correlation with isotopic labeling. Current efforts are focused on identifying the peaks of interest from both measurements.

This work is funded through NIH and NIDA award number P30 DA018310.

Keywords: Liquid Chromatography/Mass Spectroscopy, Neurochemistry, Peptides
Application Code: Neurochemistry
Methodology Code: Mass Spectrometry
Living cells communicate by vesicular delivering of chemical messengers into their environment, a process called exocytosis. Revealing transmitter storage mechanism in sub-vesicular domains is important in order to study neurotransmission and malfunction thereof in disease. As the vesicles are nanometer dimension, this is extremely challenging.

Here we use imaging mass spectrometry to study the neurochemistry in small environments of large dense core vesicles (LDCVs). The major goal is to image transmitters in the protein dense core and halo solution around it, in an attempt to measure the neurotransmitter molecule distribution between these sub-vesicular compartments – a potentially important pharmaceutical target. Our work is focused on mapping individual LDCVs in two cell models, PC12 and chromaffin cells. Cells were treated with 13C enriched L-3,4-dihydroxyphenylalanine (L-DOPA), a metabolic precursor for neurotransmitters dopamine, adrenaline and noradrenaline. 13C isotopically labeled cells were chemically fixed, for transmission electron microscopy (TEM) and following nano-scale imaging measurements. Imaging inside vesicles has been performed with a Cameca NanoSIMS 50L ion microprobe using Cs+ primary ions with approx. 50 nm spatial resolution. High spatial resolution negative secondary ion images of isotopic ratios 14N13C/14N12C and 13C12C/12C2 were acquired from micrometer-sized cell regions of the corresponding TEM sections. The overlays of isotopic ratio images and TEM images were used to measure the presence of transmitters in the vesicles as well as to reveal possible transmitter vesicular regions.

The use of NanoSIMS opens great possibilities for the study of neurochemistry in single transmitter vesicles and allows us to identify vesicle compartments as well as to relatively quantify transmitters inside them.

**Keywords:** Bioanalytical, Imaging, Mass Spectrometry, Neurochemistry

**Application Code:** Neurochemistry

**Methodology Code:** Mass Spectrometry
Mass spectrometry imaging (MSI) generates multidimensional datasets that combine spatial and chemical information. In this study, matrix assisted laser desorption / ionization time of flight (MALDI-TOF) MSI is used to investigate peptide distributions within the central nervous system of the planarian [i]Schmidtea mediterranea[/i] at different time points during animal regeneration. The planarian head is removed, and the tail is retained to monitor the regeneration of the animal’s head and brain. For MSI sample preparation, planarians at various stages of regeneration were frozen, embedded, sectioned, and rinsed in ammonium acetate buffer and isopropanol before MALDI matrix application. MSI shows many ions that localize to and around the brain in intact and mostly regenerated planarians. Several ions are also observed to localize to and around the regenerating tissue in animals that had not yet regenerated a visible brain. Chemical trends associated with regeneration are highlighted using principal component analysis (PCA). PC 1 shows a clear separation between intact and regenerating animals, and PC 2 appears to reflect animal-to-animal variations in the regeneration process. To identify the detected ions, many of which are localized and detected at low abundance, multiple extraction media were tested on homogenized tissue samples. Extracts were directly profiled via MALDI MS and then subjected to LC-MALDI MS analysis. Alternatively, micro-extractions were conducted on tissue sections to more specifically extract from the regions of interest. Our ultimate goal is to understand the space, time and chemical mosaic of the neuronal regeneration process.

Keywords: HPLC, Imaging, Mass Spectrometry, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Mass Spectrometry
Polymer electrodes have recently emerged as an alternative to traditional electrode materials like carbon or gold for the measurement of neurotransmitters. These electrodes are easy to fabricate, offer excellent electrochemical properties for the measurement of biogenic amines, and are easily incorporated into microfluidic geometries. Here, we have fabricated the first injection molded polymer microchip for the capture, stimulation, and measurement of exocytosis from single PC-12 cells. This 2-inch device was fabricated by the thermal bonding of two TOPAS cyclic olefin copolymer substrates. The top TOPAS substrate includes luer lock fittings, a serpentine channel for separating clumps of cells, and a straight channel for cell selection. The device also incorporates an auxiliary 3 micron-wide channel where suction is applied to capture and immobilize a PC-12 cell over a PEDOT electrode for stimulation and measurement. The bottom TOPAS substrate contains 500 nm cavities etched via reactive ion etching. In these cavities, a thin film of gold and chromium serves as contact leads to a 3 micron PEDOT band electrode where measurements of exocytosis from single cells or neurons can be made. We characterize the device by measuring ferrocene and dopamine using slow-scan cyclic voltammetry, and demonstrate the use of the device for easily monitoring exocytosis from single PC-12 cells.
Gathering spatially resolved chemical information is of paramount importance to understand the underlying mechanisms of different systems. As such, elemental mapping of large sample surfaces is critical in various fields, from materials science to biology. Nevertheless, low-throughput limits current techniques for elemental mapping, requiring several hours to tens of hours for obtaining a full map with sufficient pixel density. Therefore, a high-throughput technique that can achieve fast chemical imaging of large area samples is needed. Pulsed glow discharge optical emission spectroscopy (GDOES) has shown the potential to allow elemental mapping when coupled to a spectral imaging system. In this manner, it can yield quantitative-, multi-elemental mapping in seconds. Most preliminary studies have shown GDOES elemental maps of samples with medium size areas (7mm to 30mm diameter). Glow discharge cell that can accept bigger samples are required to harness the technique’s potential advantages. The development of a glow discharge chamber to accommodate samples with 100mm diameter will be presented. Furthermore, the coupling of this new GD chamber to a push-broom hyper-spectral imaging system will be discussed. Finally, novel applications enabled by GDOES elemental mapping will be shown.

**Keywords:** Atomic Emission Spectroscopy, Elemental Analysis, Imaging, Instrumentation

**Application Code:** Materials Science

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
Inorganic mercury at low concentrations does not cause significant health risks. However, its conversion to organic methyl mercury by anaerobic bacteria seen commonly in marine environments makes it extremely toxic. Methyl mercury enters the food chain through the absorption by phytoplankton and its concentration increases as we go higher in the aquatic food chain. Consumption of fish having high concentrations of methyl mercury frequently can cause health issues including chromosomal damage, reproductive damage, skin reactions etc. Hence monitoring methyl mercury levels in fish has gained significant interest.

The high demand for analyzing mercury in fish has increased the number of labs looking at direct mercury analysis since it offers significant advantages over multi-step techniques and provides a cost and time savings by eliminating digestion equipment and waste disposal.

However, the direct mercury analyzers available today are designed to analyze total mercury and not methyl mercury. In recent years, methods have been developed that allow chemists to extract the organic methyl mercury from an environmental sample and analyze aliquots of the extracted organic phase for total and speciated methyl mercury using a direct mercury analyzer. This presentation will cover:

- Introduction to direct mercury analysis and its advantages over conventional techniques
- Concept and operating principle of direct mercury analyzers
- Methods to analyze methyl mercury using a direct mercury analyzer
- Data Analysis

Keywords: Atomic Absorption, Environmental Analysis, Mercury, Trace Analysis
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Quantitative analysis in any Optical Emission Spectroscopy starts with a high level of confidence in the spectral line assignment from databases. Nevertheless, the resolution of the spectra is usually degraded by the instrument and/or the line broadening. This low spectral resolution makes it possible that spectral interferences occur for the majority of the lines. Such interference makes the elemental profile uncertain.

This presentation shows the development of a factor to quantify the level of confidence attributed to line assignment in LIBS as an example and its extension to a complete quantitative atomic profiling. This factor is combining a physical understanding of the plasma emission with a statistical analysis of the spectrum. The possible applications and outcomes of using such a quantitative factor to evaluate line assignment in plasma spectroscopy will be discussed in the context of forensic science.

The work presented is funded by the US National Institute of Justice (2012-DN-BX-K027: "Level of Confidence in Elemental Analysis by LIBS") and the State of Florida.

**Keywords:** Atomic Emission Spectroscopy, Chemometrics, Data Analysis, Forensics

**Application Code:** General Interest

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
Due to its advantages, nanotechnology has been receiving increased attention in recent literature and at current conferences. In nano, it is widely accepted that size defines properties. While this has been proven to be true on numerous occasions, it is often forgotten that impurities also define properties.

In this presentation, use of an electrothermal, near-torch vaporization sample introduction system for inductively coupled plasma-atomic emission spectrometry that allows direct (i.e., without dissolution) analysis of carbon nanotubes will be presented in detail.

Keywords: Atomic Emission Spectroscopy, Multichannel Spectrometry (CCD CID array), Nanotechnology
Application Code: Nanotechnology
Methodology Code: Atomic Spectroscopy/Elemental Analysis
There is a distinct need for portable, in field elemental analysis when rapid investigation is needed. To be portable analytical instrumentation must be small, rugged, compact, and produce minimal waste. For this purpose our laboratory has developed a liquid sampling-atmospheric pressure glow discharge (LS-APGD) microplasma that performs as an excitation/ionization source for elemental analysis. As an excitation source for analysis of small, discrete liquid samples (i.e. µL) by optical emission spectroscopy (OES), the LS-APGD operates at low solution flow rates (100-200µL min⁻¹) and low power consumption (<100 W) with He sheath gas flow rates of .6-.8 L min⁻¹ and counter flow gas rates of .2 L min⁻¹. The microplasma is generated in the 1 -1.5 mm gap between an electrode and a fixed co-linear (i.e.180 º geometry) fused silica liquid capillary that delivers an electrolytic solution sheathed in a stainless steel gas capillary delivering helium gas. The LS-APGD has been previously implemented as an ionization source for elemental mass spectrometry and for laser ablation (LA)-introduced solids and in the desorption of organic species from surfaces. The parameters of the LS-APGD have been fine tuned for OES with detection by a five channel, broad wavelength optical spectrometer, Aurora by Applied Spectra. Limits of detection, limits of quantification, robustness, working range, precision, and bias from certified reference materials have been evaluated through studies with multi element solutions. Evaluation of the key operational parameters of the LS-APGD has shown promise towards a portable, in field OES source for elemental analysis.
As miners strive to extract lower and lower levels of gold from ores (commercial mines operate with gold levels of 1 gram per ton). Fire assay has long been the go-to method for the determination of gold in ores but, as levels of gold decrease, the fire assay technique has undergone modifications in order to deliver accurate results. There is a sense amongst many analysts that the technique no longer deserves the trust it earned. In this presentation, we shall present the results of our investigations into alternative methods for the preparation of samples for the determination of precious metals by ICP optical and ICP mass spectrometry.
Biofuels are increasingly becoming an important alternative or supplement to traditional petroleum based fuels. With the new sources for producing biofuels come the potential for new contaminants not necessarily found in fossil fuels. In the Unites States, the production of biodiesel is primarily driven by the use of soy beans. Unfortunately, the competition for this raw material and others for different biofuels has had an adverse effect on the price of food stuffs produced from these crops. The search for alternative and more efficient sources of raw materials for biodiesel production is ongoing. Elemental contaminants in engine fuels such as calcium, magnesium, phosphorus, potassium, sodium, and sulfur may adversely affect the quality of the fuel, engine wear, or emissions. ASTM International has embraced ICP-OES as the approved technique for measuring most of these elemental contaminants as well as providing specifications for the biodiesel that must be met before it can be used as a fuel or blended with traditional petroleum-based diesel to form bioblends.

ICP-OES offers a rapid and multielement solution for the analysis of all 6 elemental contaminants in biodiesel. Sample preparation is quick and simple and involves nothing more than diluting the biodiesel in a solvent such as kerosene. Viscosity effects between biodiesel samples is minimized with dilution and can further be corrected with internal standards added to the solvent prior to sample dilution. With appropriate sized sample introduction hardware and plasma conditions, non-volatile solvents such as biodiesel and kerosene can be analyzed directly without the need for additional sample introduction accessories. The results obtained demonstrate that this technique is very effective and efficient for determination of elemental contaminants regardless of the raw materials used to produce the biodiesel.
Hair growth promoting agents are in high demand because of psychosocial effects of alopecia on human especially cancer patients undergoing chemotherapy. Plants have been employed since ancient times in traditional medicine for hair growth promotion. In this study, the leaf of Hibiscus rosa-sinensis a common herb in Nigeria acclaimed traditionally for hair growth-promoting potential was studied. The ethanolic extract of the leaves of H. rosa-sinensis was subjected to phytochemical screening using standard methods already adopted. The proximate compositions were determined by the official methods of analysis and the hair growth investigation of the ethanolic extract was carried using established protocols at concentrations of 2.5 mg/ml, 5 mg/ml and 10 mg/ml. Four groups of albino rats were used. The results of the phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, phenols, steroids and terpenoids. The proximate analysis showed that the leaves have high protein (48.57%) content, carbohydrate, ash, moisture, fiber and lipids were also present in descending order. The micro and macro nutrients were determined to be magnesium (91.52 mg/100g), sodium (20.40 mg/100g), iron (12.31 mg/100g), potassium (9.70 mg/100g), manganese (8.90 mg/100g), calcium (7.57 mg/100g), zinc (4.80 mg/100g), and copper (0.23 mg/100g). Complete hair regrowth was observed after 21 days treatment with the 5 mg/ml concentration of the leaf ethanolic extract, petroleum jelly was the control. The result of this study suggests that the leaf of Hibiscus rosa-sinensis has hair growth and probably hair loss prevention potentials.
Biofilms play a significant role in the emergence and proliferation of multi-antibiotic resistant bacterial strains as they protect cells from rapid exposure to antibiotics, allowing them to react to the attack by mutating at an accelerated rate or going into dormancy. Further, both physical and chemical variables affect the formation and properties of biofilms in complex and, thus far, unpredictable ways, which makes them difficult to analyze. Therefore, we are developing custom micro/nano-fluidic devices that control the formation of biofilms and combining them with surface plasmon resonance imaging (SPRi) to provide label-free, real-time, high-resolution, large-area imaging of biofilm dynamics. By changing the aspect ratio of the fluidic channels, we are able to study mature biofilms in a single plane without the need for confocal imaging.

First, Lysogeny Broth (LB) growth medium containing Staphylococcus aureus bacterial cells was loaded into the fluidic device. Next, the effect of flow rate on biofilm formation was investigated by flowing LB through the device at multiple flow rates. For biofilm removal experiments, the bacteria were allowed to grow and form biofilms under stagnant conditions, prior to the start of fluid flow. The entire process was monitored using SPRi. SPRi generates a gray scale image of the 1 sq. cm sensor surface with 10 micrometer resolution every 3 seconds. The brightness of points on the image is proportional to biomass density, allowing visualization of where cells are located on the surface.

This technique can be used for evaluating the attachment and removal of any microbial species, making it very versatile and adaptable for applications ranging from optimization of orthopedic implants to removing biofouling in chemical plants.

Keywords: Bioanalytical, Lab-on-a-Chip/Microfluidics, Method Development, Process Monitoring
Application Code: Bioanalytical
Methodology Code: Surface Analysis/Imaging
Currently a dual-luciferase reporter is used to confirm multi-miRNA regulation of mRNA. However, this sensor is limited in that it can only inspect two miRNAs at a time and only one color is used to look for both miRNA. This is a signal off type sensor that is generally less sensitive than signal on approaches. Furthermore, this biosensor is only used after a laborious process of altering miRNA expression and looking at changes in downstream products to determine which miRNA are relevant to study. We have investigated development of a mimic-mRNA sensor with a three colors each corresponding to three miRNA-mRNA binding regions. The sensor works by mimicking the sequence of mRNA of interest. Then several quenchers are strategically placed along the backbone of the mimic-mRNA. Partially complementary fluorescent reporters hybridize to the mimic-mRNA near the quenchers. The partial complementarity of the reporters will allow for a competition reaction between the miRNA analyte and the reporter for the mRNA binding spot. We will report on the design, selectivity, and sensitivity of the mimic-mRNA sensor to expedite discovery of multi-miRNA regulation of mRNA. A key attribute of this sensor is that precise knowledge of miRNA target sites on the mRNA does not need to be known.
Image processing algorithms have been developed that extract a single, numerical representation of color from digital images in the form of the hue, or H component, of the HSV color space. This quantifiable analytical parameter has been shown to be quite robust and maintains precision when obtained with different imaging devices, under varied lighting conditions, with different colorimetric indicator concentrations, and with variable sample volumes. Because this parameter is stable, simple to calculate, easily obtained without specialized equipment (images from consumer grade scanners or digital cameras are acceptable), and amenable to high-throughput analysis we have applied it to color changing assays of biological samples. We developed a hue-based pH determination method to analyze a digital image of multiple 100 [micro]l samples in a 384-well plate after addition of a universal pH indicator cocktail. We then used in-solution isoelectric focusing to determine the isoelectric point of several proteins by applying the method to quantify the pH of these small-volume fractions. We have observed a dramatic change in the pKa of some pH indicator molecules in the presence of non-ionic surfactants often used to maintain the solubility of proteins. We have developed calibration techniques and alternate pH indicator mixtures that allow the hue-based method to achieve accurate results and avoid the use of bulky, slow, and expensive pH electrodes whose surfaces can be fouled in the presence of detergents.
Whispering Gallery Mode Resonators for the Development of Label-Free Biosensing Platforms

Bioanalytical - General Interest

Light coupled into spherical dielectric resonators can exhibit resonances known as whispering gallery modes (WGM). These morphology dependent resonances occur when light traveling around the sphere returns in phase, leading to constructive interference. Efficient confinement of light is achieved when the proper resonant conditions are met which is dependent upon various factors including the effective refractive index. The ability of WGM resonators to be utilized as sensitive refractive index detectors has prompted interest in using WGM platforms for label-free biosensing. To employ WGM resonators as label-free biosensors, biological recognition elements are attached to the sphere surface. The effective refractive index is altered due to an antigen-antibody binding event, which results in a measureable WGM resonance shift. In this study, the high sensitivity, small size and flexibility of WGM microsphere resonators is explored though the development of label-free biosensing platforms. Recently, a multiplexed WGM biosensing platform was demonstrated where WGM resonances were measured through fluorescence imaging. Multiplexed detection of ovarian cancer biomarkers was achieved by imaging a field of microspheres, where the antigen identity was encoded in the resonator size. Current work focuses on taking advantage of the small resonator size by developing small volume, label-free biosensing platforms. Reducing the total volume allows for minimal sample requirements, monitoring real time binding events, and rapid analysis time. Additionally, small microsphere resonators can be easily integrated as sensitive refractive index detectors for novel imaging modalities. Progress on the development of these WGM label-free biosensing platforms will be presented.

Keywords:  Bioanalytical, Biosensors, Microscopy, Spectroscopy
Application Code:  Bioanalytical
Methodology Code:  Sensors

Session Title  Whispering Gallery Mode Resonators for the Development of Label-Free Biosensing Platforms

Abstract Title  Whispering Gallery Mode Resonators for the Development of Label-Free Biosensing Platforms

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Date:  Monday, March 09, 2015 - Afternoon
Time:  03:05 PM
Room:  277

Session #  745  Abstract #  745-5  Oral Sessions
MicroRNA (miRNA) are short, non-coding RNA strands that are involved in a wide variety of processes, such as cell-to-cell communication, gene expression and gene silencing. The under- or over-expression of various miRNA could result in a variety of abnormal states, such as cancer. As a result, being able to quantitate miRNA levels is essential to early-stage disease detection. However, a single miRNA strand could be involved in several gene expression pathways. Probing multiple strands simultaneously is needed to proper diagnosis of abnormalities. In addition, the perceived ranges of healthy miRNA levels can span several orders of magnitude, making early stage detection difficult. If the levels of miRNA bound to a particular carrier could be quantified, it could be possible to narrow the healthy range for a particular strand, and allow for earlier and more accurate diagnostics.

Asymmetrical flow field-flow fractionation, being a purely size based separation technique, is well suited to localizing miRNA to particular carriers (protein, lipoprotein complexes, vesicular) without disturbing the miRNA-macromolecular complex. A method was devised to fractionate pure serum into several fractions for miRNA localization. After extraction and quantitation, the profile of four miRNAs (associated with breast cancer) across the fractions was compared between healthy and diseased patients. The expression levels of miRNA were as expected between healthy patients and patients with breast cancer. Differences in the miRNA profile across the six fractions made it possible to determine which fractions may be most important for a particular miRNA strand.

Keywords: Bioanalytical, Biological Samples, Method Development, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Separation Sciences
Bacterial and viral infections are among the top 10 most common reasons for hospital visits [1] yet are often misdiagnosed [2]. There is a need for analytical tools which can distinguish between bacterial and viral infections as their misdiagnosis has high costs on human health. Recently, the CDC named antibiotic resistance as an immediate threat to human health and among other actions prescribed improvement of antibiotic use [3]. We report here a carbon nanotube field-effect transistor/chemiresistor for the quick, cost-effective and accurate detection of bacteria. Carbon nanotubes were immobilized through a drop-cast method onto gold-patterned Si/SiO2 substrates and functionalized with different lectins, sugar binding proteins, targeting carbohydrates present in the outer membrane of the bacteria for capture. This allows for diagnosis of infection on a broader scale. A change in device resistance indicates successful bacteria capture. Devices incubated with virus do not show a change in resistance. On the other hand, devices incubated with E. coli K12 bacteria resulted in a linear response with logarithmic cell concentration. Development of such biosensors which can distinguish between bacterial and viral infections are beneficial to decreasing the number of misdiagnosed infections and antibiotic misuse by providing an empirical test to determine infection type and screen antibiotics for efficacy.

The demand for a rapid and sensitive detection method of bacteria is growing significantly in biological, biomedical and clinical fields. Due to their shorter detection time and higher sensitivity over the conventional methods, impedimetric sensors modified with biorecognition molecules have been used for detection and quantification of bacteria. This presentation will describe the design and development of an antimicrobial peptides (AMP) based impedimetric sensor for the detection and quantification of bacteria. The sensor showed different impedimetric responses to various bacterial strains, which may relate to different affinities of the synthesized peptides for various strains. The detection limit of the sensor is $10^2$ CFU/mL for the four strains. Scanning electron microscopy (SEM) was used to further examine the binding mechanism and the morphology of the AMP modified substrate surface. The relationship between the AMP immobilization process as well as orientation of the peptides and the sensor performances will be discussed. This presentation will demonstrate the potential use of synthesized AMP in impedimetric sensors for bacteria detection.

**Keywords:** Bioanalytical, Biosensors, Biotechnology, Electrochemistry

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
A phospholipid nanogel additive was used to aid in the separation, detection and species determination of various Aspergillus species from PCR amplified clinical isolates in a rapid and portable device. Conventional methods for detection of an Aspergillus infection are cell cultures, antibody assays, imaging procedures and biopsies, which can be time consuming and may not offer species identification [1]. Aspergillus infections have a 90% mortality rate of immunocompromised or critically ill patients; therefore, accurate identification is important [2, 3]. Use of phospholipid nanogels with traditional benchtop capillary electrophoresis has determined 7 species of Aspergillus with 2 base pair resolution [3]. In a microfluidic device these additives can control valves and have been used in the separation of mannose sugars samples [4]. This unique phospholipid nanogel is thermally actuated, easily entering the channel as a liquid at 19 ºC and forming a gel in-channel at 29 ºC [4]. Unlike traditional polymer gels, this allows for quick removal and replacement at 19 ºC, and the creation of a temperature gradient of the separation matrix in the microfluidic channel [5]. Once heated in the channel the phospholipid nanogel acts as a pre-concentrator, stacking injected sample at the buffer – nanogel interface. The use of phospholipid nanogel in a microfluidic chip allows for a rapid and portable method for detection Aspergillus species with internal DNA standards, generating 550,000 theoretical plates per meter. This presentation discusses the advancement and application of nanogels for stacking, separating and determining of Aspergillus species.


Keywords: Bioanalytical, Biological Samples, Capillary Electrophoresis, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Capillary electrophoresis (CE) is an excellent analytical separation method with promising features such as small sample volume ([micro]L to pL), fast analysis time (s), high selectivity and efficiency, and excellent compatibility with biological samples. However, the inability of conventional CE detectors to sense biologically active compounds that are optically and electrochemically inactive limits their use for drug screening. We have developed a highly stable electrophysiological detection platform consisting of ion channel (IC) reconstituted in synthetic bilayer membrane or BLM across a functionalized microaperture to be coupled to a high resolution capillary separation channel. Further, the BLM platform was enhanced by > 5 fold using photopolymerizable monomers partitioned into the lipid membranes. Additionally, we have developed ion channel coupled receptor fusion protein towards wide range of ligand detection in BLM sensor platforms. The pharmacology of IC functionalized with muscarinic acetyl choline and dopamine receptors using cell based assay by patch clamp electrophysiology showed activation by acetylcholine and dopamine. Thus this platform holds a great promise as the next-generation integrated analysis system for rapid screening of biologically active compounds (eg. glucagon) in complex matrix such as whole blood and urine for the diagnosis and management of chronic disease such as diabetes.

Keywords: Bioanalytical, Biosensors, Capillary Electrophoresis, Detector
Application Code: Bioanalytical
Methodology Code: Sensors
Adipose tissue is traditionally thought to be used for storage of triglycerides, but recent discoveries suggest that adipocytes contain chemical messengers important to the endocrine system. Amino acids are thought to serve as messenger molecules in adipose tissue, much like neurotransmitters in the brain. Of particular interest are the branched chain amino acids (BCAAs): isoleucine, leucine, and valine, because evidence suggests they are highly regulated in adipose tissue. The dysregulation of these amino acids, often seen in obesity, has been correlated to the development of diabetes and other metabolic disorders. We have developed a high-speed online microdialysis assay for analyzing secretion and uptake of amino acids in adipocytes. Currently there are no adequate methods to measure these dynamics. Human pre-adipocytes, as well as 3T3-L1 cells, have been cultured and differentiated into mature adipocytes and their relative extracellular BCAA concentrations have been analyzed. Secreted amino acids are sampled using microdialysis and labeled with 4-fluoro-7-nitro-2, 1, 3-benzoazadiazole (NBD-F) in an online reaction. Microdialysis is coupled, online, to high speed capillary electrophoresis (MD-CE) and detected using laser induced fluorescence (LIF) at 488 nm, which allows for analysis of cellular secretions with a temporal resolution of less than twenty seconds. Separation conditions have been optimized for the separation of the BCAAs. The method presented allows for the detection of cellular response in response to other extracellular signaling molecules, such as glucose or insulin.

This project is funded by National Institutes of Health Grant R01-GM063533-10.
Characterized by late clinical presentation, significant co-morbidity and poor long-term survival, epithelial ovarian cancer (EOC) remains a serious medical concern to women. We have used three variants of the aptamer selection (SELEX) process to identify new functional oligonucleotides that recognize ovarian cancer biomarkers. Cell-SELEX has been used to select aptamers for cell-associated mesothelin and MUC16, two proteins implicated in metastasis and immune suppression. CE-SELEX was used to select aptamers for HE4, an informative serum marker. Finally, we have developed a novel “one-pot” selection method in which the gold standard ovarian cancer biomarker, CA25, is the target. In each case, the selection process concluded with the interrogation of the selected DNA pool by high-throughput sequencing. This sequencing process yields significantly more insight on the selection process than the conventional sequencing that has traditionally been used at the end of the SELEX process. We have developed a set of bioinformatics tools that enable the most enriched aptamer candidates to be identified. Affinity probe capillary electrophoresis, fluorescence anisotropy and label-free immunoassay platforms are currently being used to assess the affinity of the selected aptamers for their targets. We acknowledge support from the National Cancer Institute.
Chemical Cytometry of Drug Targets: Direct Quantification of Epidermal Growth Factor Receptor Activity in Single Cells

Given the clinical importance of tyrosine kinases (TKs) as drug targets in a host of human diseases, demand is high for new and improved TK assays in primary samples. Heterogeneous TK activity within populations of primary cells, and thus heterogeneous response to clinical TK inhibitors, requires direct assays of TK activity at the single cell level. We present a direct chemical cytometry assay for Epidermal Growth Factor Receptor (EGFR) TK activity and demonstrate its application in single A431 epidermoid carcinoma cells. The foundation of this assay is a short synthetic peptide substrate Htc-tide, whose phosphorylation by EGFR in single cells is quantified by single cell capillary electrophoresis with LIF detection. Htc-tide differs from traditional TK peptide substrates in the introduction of conformational constraint of the peptide backbone at the phosphorylation site. The conformational constraint is well tolerated by EGFR, but discourages assay interference by many off-target enzyme activities including protein tyrosine phosphatases and cytosolic peptidases. Dephosphorylation of phosphorylated Htc-tide by tyrosine phosphatases is significantly less efficient compared to the native tyrosine analog Tyr-tide ([i]k[/i][sub]cat[/sub]/K[i]M[/i] = 2.3 vs. 3500 for PTP1B), resulting in a major reduction of PTP assay interference. We apply the Htc-tide-based assay to directly quantify EGFR activity in single intact A431 cells and show the ability to detect fewer than 10[sup]-20[/sup] mol of phosphorylated reporter in individual cells.

Keywords: Biological Samples, Capillary Electrophoresis, Enzyme Assays, Peptides

Application Code: Bioanalytical

Methodology Code: Capillary Electrophoresis
Enzymes are used to determine the sequence of glycans. Studying glycans sequence is important because change in glycosylation pattern of glycans is the hallmark of cancer and analyzing the glycan composition is essential to therapeutics [1,2]. Neuraminidase an important enzyme used to evaluate and control sialic acid content. Traditional methods of enzyme characterization use milliliters of enzyme which require several hours for the reaction to be completed. By using capillary electrophoresis, the amount of enzyme required for analysis is reduced to nanoliter levels and enzymatic processing is complete in minutes. Phospholipid additives DMPC and DHPC were used to perform complex glycan separations [1-3]. These phospholipids self-assemble to form a thermally reversible pseudogel. Furthermore, these preparations have fluid like properties at lower temperatures and becomes viscous gel at higher temperatures [4].

Fluorophore labeling is done for the oligosaccharides to increase the charge-to-size ratio for faster separation. Neuraminidase catalytically cleaves the sialic acid from the non-reducing end of the oligosaccharide. Currently the neuraminidase being studied preferentially cleaves $\alpha_2,3$ and $\alpha_2,6$ bonds. The goal of the present study is to utilize enzyme specificity and to evaluate the catalytic efficiency of different neuraminidases.

Keywords: Capillary Electrophoresis, Carbohydrates, Fluorescence, Lipids
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Sub-micron silica colloidal crystals form uniform packing on a planar system. Eddy diffusion is greatly reduced in such uniformly-packed bed. Band broadening caused by slow mass transfer is reduced by using non-porous particles. Thus, fast and efficient electrophoresis can be achieved in silica colloidal crystals. This technique can be further improved by using label free detection. This can be achieved by in-gel protein immobilization, followed by on-column western blotting.
Subcellular liver fractions (“microsomes”) act as an excellent in vitro system to study drug and xenobiotic metabolism. Hence, developing microsomes-based green bioreactors to synthesize drug metabolites for pharmacokinetic evaluation, and microsomal biosensors for pollutant screening possess enormous significance in human health and environment. In order to achieve efficient electrocatalytic properties of microsomes, electrons should be injected to the metabolic enzymes (e.g. cytochrome P450) present in microsomes. Our goal is to drive the metabolic reactions of microsomes electrochemically by simple application of potential without requiring expensive NADPH cofactors. Our prior work identified that polished high purity graphite and edge plane pyrolytic graphite electrodes featuring high surface defects were suitable to directly adsorb microsomes with good electronic connectivity and bioactivity. In the present study, our objectives are to achieve high density immobilization of microsomal fractions to enhance product yields in the electrocatalytic conversion of drugs, and additionally offer highly sensitive biosensors. For this, we are investigating two strategies: In the first one, microsomes are bound to conductive, large surface area carbon nanostructures; in the second approach, microsomes are attached to magnetic nanomaterials and immobilized on electrodes. Direct electrochemical and electrocatalytic properties of these microsomal systems combined with nanomaterials will be discussed.

Acknowledgements: Financial support by Oklahoma State University is greatly acknowledged.

Keywords: Biopharmaceutical, Biosensors, Electrochemistry, Nanotechnology
Application Code: Pharmaceutical
Methodology Code: Electrochemistry
We work with tethered membranes which are planar supported phospholipid bilayers held above a gold electrode by a set of hydrophilic polyethylene glycol (PEG) chains covalently bonded to the gold surface by organic disulfide anchors. A lipophilic alkane phytanyl group is bonded to the top of some (typically 1 – 10%) of the PEG chains (these are the tethers) which then act as a scaffold around which the membrane lipids spontaneously cluster, eventually forming a continuous layer.

These tethered systems have been found to be good models for the study of natural cell membranes. They can accommodate both small ionophores and large ion channel proteins, and thus provide a controlled system for the study of the transmembrane ion transport. In particular it is easy to add just one type of ionophore, or protein to the system so that it is clear that results come from a single type of molecule.

We have been using an EIS (electrochemical impedance spectroscopy) method to determine membrane conductivity values. These values are particularly sensitive to the nature and concentration of ion channel or ionophore added, as well as to the presence of other membrane active species such as cholesterol.

Several case studies will be presented to highlight the versatility of the system and the insights that the EIS measurements can provide.

Keywords: Drug Discovery, Electrochemistry, Electrode Surfaces, Pharmaceutical
Application Code: Drug Discovery
Methodology Code: Electrochemistry
An Old Approach to a New Problem: Electrochemical Analysis of Hydrazine in Pharmaceuticals

Genotoxic impurities (GIs) cost the pharmaceutical industry billions of dollars a year, are carcinogenic, highly toxic and are often difficult and expensive to detect and remove from active pharmaceutical ingredients (APIs). It is imperative that new multidisciplinary approaches for detection and removal be adopted as current techniques such as mass spectrometry and high pressure liquid chromatography are labor intensive and suffer from long analysis times. In this work, a flow injection analysis approach with electrochemical detection is utilized for hydrazine quantitation in the presence of excess electrochemically active APIs. It is possible to screen out the interfering effects from APIs via functionalization of a polycrystalline boron doped diamond microband electrode with metallic nanoparticles as shown in Figure 1.

Coupling of this electrode with FIA produces a tuneable sensor with high signal to noise ratio, fast data acquisition and small sample requirement. Our results demonstrate hydrazine quantitation down to 64.5 nM (274 ppb) in the presence of excess acetaminophen (50 mM), surpassing the limits of detection required by pharmaceutical guidelines, without the need for excessive sample preparation or derivatization.

**References**

**Keywords:** Electrochemistry, Flow Injection Analysis, Lab-on-a-Chip/Microfluidics, Pharmaceutical
According to the 2012 statistics from the American Diabetes Association, 9.3% of the population in the United States suffer from diabetes. This metabolic disorder arises either from insulin deficiency (Type-1, insulin dependent) or insulin resistance (Type-2, insulin non-dependent). Insulin, a polypeptide hormone, maintains glucose homeostasis and its concentration levels (picomolar) in the body fluids can help in diagnosing the type of diabetes. Our objective is to develop a sensitive, simple electrochemical quartz crystal immunosensor for detecting insulin levels in clinical samples. We have devised magnetic nanoparticle-antibody-antigen bioconjugates to accomplish direct diabetes relevant picomolar insulin detection in whole blood by a sandwich immunoassay. The sensor outputs are based on changes in oscillation frequency and double layer capacitance. The designed immunosensor enabling dual mode measurements adds good sensitivity and reproducibility for detection of insulin in a complex biological fluid and thus presents a promising platform for clinical applications.

Acknowledgements: This work was supported by the National Institute of Diabetes And Digestive and Kidney Diseases of the National Institutes of Health.

Keywords: Biosensors, Electrochemistry, Immobilization, Immunoassay
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Nitrogen-doped carbon nanotubes (N-CNTs) provide an excellent platform for the electrochemical detection of enzymatically generated hydrogen peroxide. Glassy carbon electrodes were modified with 7.4 wt% N-CNTs and used to immobilize glucose oxidase (GOx) through spontaneous adsorption; the enzyme’s catalytic rate was then detected through amperometric oxidation of the enzyme catalyzed H₂O₂ byproduct. Inhibition of GOx by heavy metals ions such as Ag⁺ was detected by percent decrease in amperometric current down to a 0.19 ± 0.04 ppb limit of detection (3) in phosphate buffer solution. The Ag⁺ sensor displayed a sensitivity of 2.00 x 10⁸ M⁻¹ at a low operating potential of 0.05 V (vs. Hg/HgSO₄), a linear range of 20 - 200 nM, and sample recovery at 101 ± 2%. Interestingly, the sensor experienced no loss in sensitivity with continued use; due to the %inhibition based detection scheme, the loss of enzyme with each use is inconsequential. Cu²⁺ and Cd²⁺ were found to be much less inhibitory with sensitivities of 1.45 x 10⁶ M⁻¹ and 2.69 x 10³ M⁻¹, respectively. The inhibition constants (Kᵢ) and modes of inhibition were determined for both Ag⁺ and Cu²⁺, and a strong correlation between Kᵢ and sensor sensitivity was found.

Keywords: Biosensors, Electrochemistry, Environmental Analysis, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Nanomaterials have received much attention for their outstanding catalytic activity, large surface area, and excellent photonic and electronic features. These characteristic properties have been used to improve the sensitivity and the specificity of biosensors. In our work, we used advanced nanomaterials such as graphene, quantum dots and gold nanoparticles to assemble on the surface to construct new biosensors for the determination of biomolecules and tumor cells. Based on the above principles, a new electrochemical assay strategy was proposed based on the specific recognition of folate receptors on a cell surface to folic acids CdSe/ZnS quantum dots (QDs)-labeled folic acids (FAs) can couple with the folate receptors (FRs) on the KB cells, which can overexpress FRs. By square wave stripping voltammetry (SWSV), the developed electrochemical assay showed an excellent analytical performance for the sensitive detection of KB. We also proposed a novel and sensitive label-free aptasensor has been developed based on direct electrodeposition of gold nanoparticles (AuNPs) on NH2-graphene modified glass carbon electrode for the determination of adenosine triphosphate (ATP). These deposited AuNPs produced a sharp electrochemical current peak, which can be used as probes for the detection of ATP. The graphene/AuNPs based-aptasensor is sensitive, selective and stable and has a wide linear range from 10 pM to 100 nM, with the detection limit of 10 pM. These proposed sensing strategies should offer a new way for directing the development of an electroanalytical chemistry and will have the potential applications in clinical diagnostics and biotechnology.

**Abstract Text**

Nanomaterials have received much attention for their outstanding catalytic activity, large surface area, and excellent photonic and electronic features. These characteristic properties have been used to improve the sensitivity and the specificity of biosensors. In our work, we used advanced nanomaterials such as graphene, quantum dots and gold nanoparticles to assemble on the surface to construct new biosensors for the determination of biomolecules and tumor cells. Based on the above principles, a new electrochemical assay strategy was proposed based on the specific recognition of folate receptors on a cell surface to folic acids CdSe/ZnS quantum dots (QDs)-labeled folic acids (FAs) can couple with the folate receptors (FRs) on the KB cells, which can overexpress FRs. By square wave stripping voltammetry (SWSV), the developed electrochemical assay showed an excellent analytical performance for the sensitive detection of KB. We also proposed a novel and sensitive label-free aptasensor has been developed based on direct electrodeposition of gold nanoparticles (AuNPs) on NH2-graphene modified glass carbon electrode for the determination of adenosine triphosphate (ATP). These deposited AuNPs produced a sharp electrochemical current peak, which can be used as probes for the detection of ATP. The graphene/AuNPs based-aptasensor is sensitive, selective and stable and has a wide linear range from 10 pM to 100 nM, with the detection limit of 10 pM. These proposed sensing strategies should offer a new way for directing the development of an electroanalytical chemistry and will have the potential applications in clinical diagnostics and biotechnology.
The multifaceted environs in which cells interact are highly differentiated, and susceptible to extreme changes that require cellular survival, division, differentiation, or death. These responses are not limited to only one cell, but are usually a group response, dictated by the change in signal molecule concentration. Therefore, extracellular molecular signaling is paramount to the survival and destruction of cellular organisms. Yet, there is a shortage of methods for the direct, in vitro correlation between observed changes in cell behavior and proliferation to extracellular molecular signal (ECMS) concentration. This is subsequently due to the lack of small scale culture methods needed to confine cells to isolated small volumes (pL-µL) to explore spatial and populations/density relationships within/between cellular colonies, and the low detection limits required for valid results.

Efforts toward quantification of ECMS concentration relative to observed cell behavioral changes within microenvironments via electrochemistry are described. The electrode material is a carbon based optically transparent electrode made from pyrolyzed photoresist film (PPF). The strategies discussed include using a laser based technique to photocrosslink a protein fabrication solution into µ3D-protein hydrogel structures around cells, confining cells within 3D volumes on the electrode surface, and micropatterning the electrode to improve detection limits for ECMS target molecules. The electrode will be used to make in vitro voltammetric and amperometric measurements of cell colonies isolated within 3D structures. Fluctuations of the electrochemical signal combined with optical microscopy could link the electrochemical signal generated from a specific signal molecule to observed cellular behavior in real time. Target molecules discussed include nitric oxide, NADH, hydrogen peroxide, and oxygen.

Keywords: Biomedical, Biosensors, Electrochemistry, Microelectrode
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Decreasing Identification Time for Pseudomonas Aeruginosa Using a Simple Electrochemical Sensor

Certain virulence factors, produced by bacteria, can be sensed via electrochemical detection. Detection of these factors can be used for rapid differential diagnosis of bacterial infections, leading to the application of targeted antibiotics. Here we report the selective and sensitive detection of pyocyanin, uniquely produced and excreted by the opportunistic pathogen Pseudomonas aeruginosa, using unmodified carbon electrodes embedded in Kings A agar. Compared to visual inspection, which usually requires 24-72 hours for identification, electrochemical sensing allows conclusive identification, starting from as few as 100 cells, in less than 18 hours. The identification time can be reduced further by optimizing the plate geometry and improving electrochemical sensitivity.

Disposable screen printed electrodes were embedded into culture plates, by pouring sterilized King’s A agar over them. King’s A agar is known to increase the production of pyocyanin. Square wave voltammetry (SWV) scans of the virulent P. aeruginosa strain PA14 were taken every hour. Optical images of the cultures were collected to determine when P. aeruginosa could first be detected visually.

In the potential range from -0.5 to 0 volts, no molecules other than pyocyanin (in P. aeruginosa cultures only) were detected. To confirm selectivity, SWV scans were obtained from Staphylococcus aureus, one of the most common pathogens. Temperature dependent production of pyocyanin was observed, with the highest production rates and earliest detection times for P. aeruginosa at temperatures above 37 degrees Celsius. The results indicate the potential for sensitive and selective electrochemical determination of P. aeruginosa infection directly from patient samples. By integrating detection with a commonly accepted format, we hope that this technique will be rapidly adopted and utilized by the healthcare industry.

This work was supported by the U.S. National Science Foundation under Grant No. 1125535.

Keywords: Bioanalytical, Clinical Chemistry, Electrochemistry, Voltammetry
Application Code: Clinical/Toxicology
Methodology Code: Electrochemistry
A Comprehensive Removal Study of Selected Pharmaceuticals by PACs in Source and Drinking Water Using LC-MS/MS

Pharmaceuticals and personal care products (PPCPs) are used for a wide variety of applications. U.S. Geological Survey published a screening study results in water in 2002. Detectable PPCPs were found in 110 out of 139 susceptible streams in 30 states in USA. Due to large amount of PPCPs that may have been transported to natural water resources, the removal of PPCPs in source water and drinking water becomes important.

An Ultra-fast and sensitive UFLC-MS/MS method was developed for the detection of 6 selected PPCPs, including azithromycin, cephepirin, enrofloxacin, ciprofloxacin, diphenhydramine and cotinine. Different powdered activated carbons (PACs) including Super activated carbon nano powders (raw material coconut) Aqua Nuchar (AN) and Hydrodarco B (HB) were used to remove the selected PPCPs. Different pHs water matrices, dosing concentrations, and contact times were evaluated. HB at the dosage 2 mg/L removed over 60% of azithromycin, cephepirin, enrofloxacin, ciprofloxacin and diphenhydramine have over 60% removal after 4 hours contact time. For AN, the removal trend of the PPCPs was the same for most of the selected PPCPs but with higher removal efficiency except cephepirin compare with HB. Cotinine could not been removed by any of the tested PACs under the experimental conditions in this study. The detailed experimental conditions, interferences, and results will be presented at the conference.

This research was supported by Missouri Department of Natural Resources.

Keywords: HPLC Detection, Mass Spectrometry, Water
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
Disinfection byproduct anions, such as chlorite, bromate, and chlorate are predominantly produced from chemical disinfectants used to treat potable water systems. Bromate is a byproduct from the ozonation of waters containing bromide. The U.S. EPA listed bromate as a probable human carcinogen at low ppb concentrations. Based on available toxicology data, the U.S. EPA has specified a maximum contaminant level (MCL) of 10 ppb for bromate and 1000 ppb for chlorite. In a previous study, we demonstrated the advantages of use a hydroxide-selective anion exchange column for the determination of bromate and other oxyhalides, such as a lower background, lower noise, and therefore lower detection limits relative to using a carbonate eluent that is described in U.S. EPA Method 300.1 (B). Additional improvements in column and suppressor technologies over the last several years has enabled even lower detection limits and better resolution of oxyhalides and bromide in drinking water samples. In this report, we demonstrate this determination using a recently developed hydroxide-selective column combined with electrolytically regenerated suppressed conductivity detection. The calculated method detection limits for chlorite, bromate, chlorate, and bromide in this study were determined to be 0.1, 0.12, 0.14, and 0.21 ppb, respectively. To determine the accuracy of the methods, drinking water samples were spiked with known concentrations of oxyhalides and bromide, which produced recoveries ranging from 83-92%, 93-108%, 93-101%, and 91-100% for chlorite, bromate, chlorate, and bromide, respectively. The linearity, accuracy, and precision for the determination of oxyhalides and bromide will be discussed further in this report.
Abstract Text
The photoionization detector (PID) is a very sensitive GC detector for sulfur compounds with pg detection limits for hydrogen sulfide. This compound can be efficiently collected in the field in an impinger with a basic solution (0.01M NaOH) at a known period of time and flow rate. The solution should be kept out of sunlight. Back in the lab, since the pKa for H2S is 6.9, the addition of 0.1 M acid will convert the sulfide (collected) to H2S which can be swept out of a vessel with an inlet, exhaust and septum (for addition of acid {H+}). Once the H+ is added, the solution is stirred for several minutes, then the nitrogen is turned on at 15 cc/min/ and the H2S is swept into the sample loop of the six port GC injection valve. The H2S is separated on a porous polymer column and detected by the PID. A 10ng/L sample (permeation tube) of H2S collected for 10 minutes indicated a detection limit (3 sigma) of 0.9 ppb. The coefficient of variation at 25 ppb was 16.3% for 5 successive runs. The PID has a dynamic range > 107 so a high level stack sample (200 ppm) would still be in the linear range with a slightly shorter sampling time.

Keywords: Environmental Analysis, Gas Chromatography, GC Detectors, Specialty Gas Analysis

Application Code: Environmental

Methodology Code: Gas Chromatography
In indoor air measurements, volatile organic compounds (VOCs) are major hazardous pollutants originating from emission of building materials. An important group is the semi-VOCs (SVOCs). These components, such as the phthalates, have boiling points between 240-260°C to 380-400°C (ISO 16000-61) and low vapour pressure. To guarantee the Quality Assurance and Quality Control (QA/QC) and therefore the accuracy of their measurement, gas standards in the form of transfer reference materials are necessary.

This presentation gives an overview of the recent developments and challenges in the preparation of a selection of VOC and SVOCs gas standards at trace levels (few ppb levels) and their loading into sorbent tubes. In addition, details of their analysis by will be showed.

The preparation consists of dynamic blending of vapours of VOCs and SVOCs with clean air followed by pumped sampling of know volumes of this gas mixture into thermal desorption type tubes. During the study, the loading levels varied from 30 to 500 ng of respectively styrene, n-hexadecane, dibutyl phthalate, dimethyl phthalate, 2-ethyl-1-hexanol, 1-methyl-2-pyrrolidone. The method of analysis of the standards tubes consisted of thermal desorption followed by GC-FID analysis.

The results of the study show that it is possible to obtain SI traceable gas standards at trace levels with an expanded uncertainty between 3 and 5 %. This research was part of the MACPoll project under the European Metrology Research Programme.

Keywords: Reference Material, Semi-Volatiles, Thermal Desorption, Volatile Organic Compounds
Application Code: Environmental
Methodology Code: Gas Chromatography
Environmental - LC/GC Techniques
Separation of Uremic Toxins with a Resorcinarene-Based IC Column

People with chronic kidney disease suffer from the uremic toxins which accumulate in their bodies. Detection and quantification of the uremic toxins helps diagnose kidney problems and start patient care. The aim of this research was to achieve trace level detection and separation of uremic toxins in water and urine. Some uremic toxins contain guanidino groups, which in neutral aqueous environments are be protonated. To detect and quantify the guanidine containing uremic toxins, new stationary phases for ion chromatograph (IC) columns were prepared. The separation materials were based on glutamic acid functionalized resorcinarenes (GAU) bound to a 4.6 µm divinylbenzene macroporous resin packed in a 3 × 150 mm PEEK column. Two different packing methods, one using sonication and the other using grinding, afforded fast and efficient separation of the five compounds: guanidineacetic acid (GAA), guanidine (G), methylguanidine (MG), creatinine (CRN), and guanidinobenzoic acid (GBA). Isocratic and gradient elutions with methanesulfonic acid (MSA) solution as eluent resulted in fast separation of guanidino compounds on packed GAU column. The column efficiencies, reported as the number of theoretical plates which shows significant plates number for our designed IC column. The peak resolutions at room temperature and elevated temperatures were calculated and reported. Trace levels of analyte were detected directly by electrochemical detection with a gold working electrode in urine sample. Quantitative calibration showed linear curves for the gold working electrode in water, urine and elevated temperature. Pulsed amperometric detection method successfully measured analyte concentration from 30 to 158 ppb in water and 93 to 591 ppb in urine at room temperature.

Keywords: Biological Samples, Electrochemistry, Ion Chromatography, Ion Exchange
Application Code: Environmental
Methodology Code: Separation Sciences
The toxicity, microbial resistance, and unforeseen adverse effects in the environment, induced by pharmaceuticals, necessitate a comprehensive study of abatement, degradation pathways, and toxicity changes of each target pollutant during advanced oxidation processes (AOPs). The selected AOPs included UV-based and O₃-based (e.g. UV, UV/H₂O₂, and O₃/H₂O₂) technologies, which have shown great potential for effectively removing pharmaceuticals from sewage effluent. Six frequently detected pharmaceuticals in sewage effluent, including sulfadiazine (SDZ), norfloxacin (NOR), trimethoprim (TMP), metoprolol (MET), roxithromycin (ROX), and caffeine (CAF), were selected for this study. The removal efficiency and degradation kinetic studies were conducted using liquid chromatography tandem mass spectrometry (LC-MS/MS). The abatement and degradation byproducts of these pharmaceuticals by the selected AOPs were studied using liquid chromatography time-of-flight mass spectrometry (LC/Q-TOF-MS) to propose degradation pathways. In addition, this project has evaluated the pollutant removal efficiency, energy consumption, and operation factors of the different AOPs and determined the most applicable method. This research was supported by National Science Foundation East Asia Pacific Summer Institutes (Grant #1414954) in collaboration with the Chinese Ministry of Science and Technology, and the National Natural Science Foundation of China (Grant #51221892).

Keywords: Environmental/ Water, Liquid Chromatography/ Mass Spectroscopy, Pharmaceutical, Time of Flight M
Application Code: Environmental
Methodology Code: Liquid Chromatography/ Mass Spectrometry
Environmental - LC/GC Techniques

Anion and Cation Analysis of Produced Water From Hydraulic Fracturing Using Ion Chromatography

The increase in U.S. natural gas production can be largely attributed to the extensive use of hydraulic fracturing (also known as fracking). This process extracts natural gas and oil by drilling into bedrock (primarily shale) several thousand feet below the surface and then injecting fluid under high pressure causing cracks to form. As a result, trapped oil and gas is released that can then be recovered. Hydraulic fracturing requires large quantities of water, putting stress on local sources, and produces large amounts of wastewaters, which must be either disposed of or reused. Treatment and reuse has significantly reduced the water and disposal requirements of this process. Knowing the composition of ions in wastewater can be used to develop effective treatment strategies and optimize fracturing fluids that are created from this water. Additionally, this information can be used to meet stringent local reporting requirements for fracturing activity.

This presentation describes the use of ion chromatography (IC) to determine anions and cations in produced water from three different hydraulic fracturing sites. Considerable variation in ion concentration was found, which was attributed to differences in the geology of the locations from which samples were obtained.

Abstract Text

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Keywords: Analysis, Environmental/Waste/Sludge, Fuels\Energy\Petrochemical, Ion Chromatography

Application Code: Environmental

Methodology Code: Liquid Chromatography
This research presents the use of a low detection limit GC for field investigation of individual VOC time-dependent concentration trends in residential areas near a petrochemical industrial park at central Taiwan. The prototype GC is previously developed in our group which equips a multi-stage mini-preconcentrator with amplification factor > 10,000, a capillary column with at-column heater configuration, and a photo-ionization detector (PID). An in-lab calibration shows that detection limit of GC is 0.02 ppb for benzene. A tablet computer was embedded inside the GC which enables the system to be remotely controlled and read out by a cell phone via wireless internet. The GC weighs less than 3 kg and consumes only 15 W on average during analysis. The field study data was obtained by using the GC continuous analysis (24 hr) at two different sites (school and residence) located approximately 3 km away from a petroleum chemical industrial area. Side-by-side canister/GC-MS was used to provide qualitative reference. Six VOCs including acetone, chloromethane, methyl t-butyl ether, hexane, toluene, m/p-xylene were found at first site. Three VOCs including, acetone, 1,3-pentadiene, toluene were found at second site. The concentration range varied from 0.5 to 4.8 ppb for most VOCs. The chemicals resulted from either traffic sources or industrial area can be seen with different time patterns. This newly developed GC enables continuous and near-real-time VOC analysis at sub-ppb level with very low cost per analysis which can be useful for tracking time-dependent trends for individual pollutants.
Between July 9 – 12, 1997, at least 400 tonnes of polyvinyl chloride (PVC) were consumed in a fire at the Plastimet Inc. plastics recycling facility in Hamilton, Ontario, Canada. This led to the release of contaminants, including highly toxic polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF). This study re-examines a composite soil sample collected shortly after the fire using state-of-the-art FT-ICR (Fourier transform ion cyclotron resonance) and GC×GC-TOF (comprehensive two dimensional gas chromatography-time of flight) mass spectrometry. The FT-ICR experiments led to the identification of approximately 150 molecular formulae, corresponding to chlorinated and mixed chloro/bromo compounds. The majority of these are halogenated polycyclic aromatic hydrocarbons (halo-PAHs), including highly substituted (e.g. C14HCl9 and C16HCl9) and high molecular weight (e.g. C28H12Cl4) Cl-PAHs that have not been reported previously in environmental samples. Complementary GC×GC-TOF experiments resolved individual halo-PAHs, some of which were confirmed with available standards. The concentrations of the most abundant halo-PAH groups, C14H8Cl2 (22 µg/g) and C16H8Cl2 (20 µg/g) are much higher than reported dioxin values and comparable to the corresponding PAH groups C14H10 (12 µg/g) and C16H10 (19 µg/g). The high abundance of the halo-PAHs identified in this study highlights the need for further investigation into their environmental occurrence and risk.

Keywords: Environmental Analysis, Gas Chromatography/Mass Spectrometry, PAH, Volatile Organic Compounds
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
The objective of this research is to develop an analytical method for the identification of emerging contaminants of concern, such as bio-terrorism analytes, illicit drugs and metabolites and other toxic compounds. Due to the wide range of compounds of interest, the methodology of analysis must be able to successfully characterize compounds in a complex sample. In order to achieve this level of sensitivity and selectivity, both Comprehensive Gas Chromatography coupled with Time-Of-Flight Mass Spectrometry (GC x GC-TOFMS) and High Performance Liquid Chromatography couple with Time-Of-Flight Mass Spectrometry (HPLC-TOFMS) were utilized to analyze wastewater samples obtained from the Pennsylvania State University wastewater treatment facility (WWTF). The ultimate goal is to determine emerging contaminants and define temporal and spatial characteristics of usage at the community level.

In addition to the development of a method for the identification of contaminants, the environmental transport and fate of identified contaminants was investigated to determine the potential implications of identified compounds. Plant and soil samples from the Pennsylvania State University agricultural fields, which use an open-loop water system, were collected to refine analytical methodology. Multiple water samples were gathered from different stages throughout the Penn State WWTF and treated with USEPA method 3510c, which uses a liquid-liquid process and Kuderna-Danish to concentrate samples to 1 mL. The normalized background developed in earlier stages was used to identify any new contaminants emerging in the wastewater samples.

Keywords: Environmental/Water, Forensic Chemistry, GC-MS
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Forensic investigation of hazardous substances is essential for effective response to chemical spill emergencies or other accidents. Rapid and accurate identification of unknown emergency samples is always a challenge because there is usually little background information available to select the appropriate sample preparation and analytical methodology. In addition, the samples are often chemically complex and have been altered by the environment, and particularly, the potential target compounds are often co-eluted with interfering substances during an instrumental analysis. At present, the identification of the organic substances is mainly based on information from analyses by gas or liquid chromatography coupled with mass spectrometry (GC/MS). However, normal low resolution single quadrupole MS typically provides less conclusive information. At the same time, time-consuming extraction and cleanup are not suitable for the rapid analysis of emergency samples.

This work presents our studies on the application of the latest chromatographic and spectral technology in identifying unknown hazardous substances. The Agilent 7200 gas chromatography coupled to an accurate-mass quadrupole time-of-flight (GC/Q-TOF) mass spectrometer inherits both advantages of powerful separation of GC and rapid and accurate TOF detection. GC/Q-TOF simultaneously provides a good tool for solving complex analytical problems, resolving structural elucidation of unknowns, and confirmation of non-target compounds. Preliminary results demonstrate that accurate mass information not only enables a rapid and efficient screening of target and non-target compounds and increases detection selectivity, but also improves the quantitation confidence by eliminating those interferences presented with nominal mass chromatograms.

**Keywords:** Chromatography, Environmental Analysis, Forensic Chemistry, Gas Chromatography/Mass Spectrometry

**Application Code:** Environmental

**Methodology Code:** Gas Chromatography/Mass Spectrometry
There are several Environmental Protection Agency (EPA) volatile methods. The sample introductory technique specified by the EPA is Purge and Trap (P & T). This presentation will focus on enhancements to these methods utilizing new P & T techniques and advancements in Gas Chromatography/Mass Spectrometry (GC/MS) technology.

In addition to providing improved productivity for the industry, the results attained using alternative carrier gases such as hydrogen and nitrogen will be compared to helium. This is advantageous due to the difficulty of obtaining helium, and the exorbitant price of helium, the current carrier gas, used for this method.

Improvements to analytical parameters, in addition to comparing results from alternate carrier gases, will be discussed.

Keywords: Environmental, Gas Chromatography/Mass Spectrometry, Purge and Trap, Water
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
In the summer of 2009, the FDA obtained authority to regulate the manufacturing, distribution, and marketing of cigarettes and smokeless tobacco products. The Family Smoking Prevention and Tobacco Control Act was passed to reduce the trend of new users forming an addiction to tobacco before they were of the age to have an understanding of the toxic and even deadly consequences. However, other tobacco products, such as hookah apparatuses, water pipe tobacco, e-cigarettes, and other tobacco or tobacco free alternatives were not controlled until very recently, April 24th, 2014, when the FDA announced they were in the beginning phases of supervising the new alternative cigarette niche.

Shisha Steam Stones are one alternative tobacco product the CDC currently admits to having no available research. These small pebble-like rocks are coated in a viscous flavored formulation, consisting mostly of glycerin, which is placed in a hookah head as an alternative to hookah tobacco. In order to analyze for the volatile organic compounds in hookah tobacco smoke, solid phase micro extraction (SPME) was used in tandem with gas chromatography mass spectrometry (GCMS). A smoking machine was constructed to mimic a hookah apparatus, where the smoke passed into miniature cold vacuum trap suspended in a dry ice + acetonitrile cold bath at -40°C and collected in NMR vials cut to a length of 2 centimeters. The VOCs found in tobacco and steam stone smoke differed substantially, specifically with the absence of nicotine and other toxic compounds in the steam stone smoke.

**Abstract Text**

In the summer of 2009, the FDA obtained authority to regulate the manufacturing, distribution, and marketing of cigarettes and smokeless tobacco products. The Family Smoking Prevention and Tobacco Control Act was passed to reduce the trend of new users forming an addiction to tobacco before they were of the age to have an understanding of the toxic and even deadly consequences. However, other tobacco products, such as hookah apparatuses, water pipe tobacco, e-cigarettes, and other tobacco or tobacco free alternatives were not controlled until very recently, April 24th, 2014, when the FDA announced they were in the beginning phases of supervising the new alternative cigarette niche.

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Significance of the Research: Ambient air is polluted by many VOCs coming from Petrochemical Industry. PAMS, Oxygenated or Halogenated VOCs can be found in many ranges of concentration between background and site fence line. These compounds are precursors of ozone and Industrial bodies need to identify them to be in compliance with the authorized limits and to improve their process.

Objectives: Chromatotec is developing a system capable of measuring online and in field conditions a large number of VOCs from ppt to ppm. The device is required to identify automatically potential coeluted compounds by MS technology adapted to industrial context.

Solution adopted: The coupling of two different FID GCs to a Quadrupole MS allowed by an elaborated multiplexer system is the originality of the project: one TDGC for light compounds and one TDGC for heavy compounds with specific trapping conditions and variable sampling volumes.

Results: A measurement campaign in petrochemical new site shows the concentration of around 100 compounds at different steps of the commissioning. Variations during specific hours are detected. Potential coeluted compounds as terpenes or organochlorinated are identified and monitored at ppt level.

Conclusion of the project: The ability of coupling in continuous two different GCs to a unique MS and the automatic identification is a new advance in the technology of industrial GC-MS. The big advantage is the possibility to play with two different trapping and thermodesorption techniques linked to one MS. This fully automatic system allows non-specialist operators to access to expertise level results.

Keywords: Environmental Analysis, Fuels\Energy\Petrochemical, Gas Chromatography/Mass Spectrometry, Volatile Compounds
Benzene, toluene, ethylbenzene, xylenes, and styrene (BTEXS) are volatile organic compounds (VOCs) associated with adverse health effects that include damage to the kidneys and central nervous system, and are known carcinogens and mutagens. The presence of these compounds in blood can result from exposure to consumer products, polymers, paints, adhesives, fuel, emissions, and smoke. Concerns about BTEXS exposure from predominant sources (fossil fuel products and cigarette smoke) have led to regional and national biomonitoring studies that characterize exposure levels. The ability to distinguish BTEXS exposure from various sources is not only important for identifying unsuspected sources, characterizing remediation efforts, and understanding public health, but also important for accurate quantification of various sample matrices as BTEXS are prevalent in common laboratory supplies (e.g., butyl rubber, helium, and acetylene). In this method, whole blood samples are collected in specially cleaned, VOC-free blood collection tubes and prepared by homogenization with isotopically labeled analog internal standards to compensate for loss biases and competition effects. Blood samples are quantified down to low ppb levels using passive headspace sampling by SPME followed by GC/MS-SIM configured with cryofocusing. Once laboratory biases are controlled, it is possible to distinguish and accurately quantify exposure levels from other different sources. In this work we compare inter-laboratory results that are biased by differences in laboratory materials used, and describe how to distinguish exposure sources among different regional and national biomonitoring studies.
Negative impact of aromatic amines and amides to the health of general population led to a ban of some of them in cosmetology, textile industry and agriculture. Therefore there is a demand in the development of rapid and reliable methods for the detection of aromatic amines and amides in the environment. GCMS methods are the most efficient in structure elucidation of these chemicals when examined as chemical modification products. We have undertaken a task of developing dependable GCMS methods for their structure elucidation. The objects of the study were isomeric hydroxyl-, mercapto- and amino-anilines, various diphenyl and triphenyl-amines, phenyl-naphthyl-amines and amides of benzoic acids.

General dissociation directions for N-alkyl, -trifluoroacetyl, -chlorodifluoroacetyl, -pentafluoropropionyl, -heptafluorobutyryl and -trimethylsilyl derivatives of aryl-amines and -amides will be presented. This will include comparative analysis of spectra for various derivatives. Emphasis will be made on the specific fragmentation pathways useful for structure determination by GCMS: in the series of mono-perfluoroacyl amines para-isomers show dramatically different fragmentation directions that allows their reliable detection; di-substituted perfluoroacylamines can be successfully used for differentiation of [i]ortho[/i]-isomers from their [i]meta[/i]- and [i]para[/i]-analogs; mixed derivatives, such as N-methyl-N-acyl, are good for differentiation of aryl moieties and the positions of amino functions. Hydrogen and oxygen rearrangement processes in the case of perfluoroacylarylamines are recognized and the fragmentation mechanisms are established.

As a result, diagnostically important ions for differentiation of isomers are identified and an effective approach for the detection of aromatic amines has been developed.

Keywords: Derivatization, Environmental Analysis, Gas Chromatography/Mass Spectrometry
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Extending the Range of Compounds Amenable for GC-MS Analysis

The major shortcoming and Achilles Heel of GC-MS is its inability to analyze relatively non-volatile and thermally labile compounds. We found that the GC elution temperatures can be significantly lowered upon the reduction of the column length, increase of carrier gas flow rate, reduction of capillary column adsorption film thickness and lowering the temperature programming rate. Over 200 degrees lower elution temperatures were achieved with our GC-MS which is equipped with a supersonic molecular beams (SMB) (also named GC-MS with Cold EI) system. Furthermore, via using high column flow rate during the injection, lower injector temperatures can be used and sample degradation at the Cold EI fly-through ion source is inherently eliminated.

The resulting lower elution temperatures in various analyses is further complemented via the feature of enhanced molecular ions obtained with GC-MS with Cold EI combined with the elimination of ion source related peak tailing and degradation. Thus, the reduced GC separation power involved in the analysis of thermally labile and low volatility compounds is compensated by increased MS separation power.

The extension of the range of compounds amenable for GC-MS analysis will be demonstrated with the 5975-SMB GC-MS with Cold EI in the analysis of large hydrocarbons up to C74H150, large polar drugs such as Reserpine, thermally labile explosives such as TATP, PETN, Tetryl and HMX, thermally labile carbamate pesticides such as methomyl, aldicarb, aldicarb sulfone and oxamyl and a range of free fatty acids.

Consequently, GC-MS with Cold EI bridges the gap with LC-MS.
Development of a Soft Ion Source for GC Used with a High Resolution TOFMS

Utilization of alternative ion sources for GCMS which generate primarily the molecular or quasi molecular ion, commonly known as soft ionization, has seen increased interest in the past few years. The combination of soft ionization and high resolution MS allows nearly unambiguous molecular formula identification; information that is extremely valuable when performing unknown compound identification.

In this work we use a vacuum ultraviolet (VUV) lamp and a dopant compound to generate a plasma within our ion source. Once this plasma is formed, ionization of organic compounds can take place in a variety of pathways generating primarily molecular or quasi molecular ions. We have used this ion source to couple a GC to an ultra high resolution TOFMS with mass spectral resolving power in excess of 50,000@FWHM. This source provides soft ionization relative to an EI source, conventionally used for GCMS. The advantage of soft ionization is that molecular ion information is retained and, when coupled with ultra high resolution TOFMS, high mass accuracy is achieved which can be a powerful tool for compound identification. High resolution also assists in retaining isotopic fine structure when multiple ionization pathways are present, further assisting in compound identification. In addition, rapidly pulsed post source fragmentation can be done enabling quasi-simultaneous collection of precursor ion spectra and fragment ion spectra across the entire chromatogram. Results demonstrating this capability will be shown for a variety of compounds.

Keywords: Gas Chromatography/Mass Spectrometry, Instrumentation, Mass Spectrometry, Time of Flight MS
Application Code: General Interest
Methodology Code: Gas Chromatography/Mass Spectrometry
The possibility of converting various biomass feed stocks directly to biofuels or value-added specialty chemicals using catalytic pyrolysis has precipitated a demand for a fast, reliable method to characterize newly developed catalysts. Catalytic pyrolysis is a three-step process: (1) the feedstock is first pyrolyzed (often referred to as “fast pyrolysis”), (2) the pyrolyzates flow through a catalyst bed and (3) the ‘products’ are identified and quantitated. The Tandem Micro-Reactor GC/MS system integrates these three processes into a single instrument.

The Tandem Micro-Reactor is designed for the rapid evaluation and characterization of catalysts in various atmospheres, at different temperatures (up to 900°C) and under different pressures (up to 3.5 MPa [500 psi]). The system consists of an upper micro-reactor and lower micro-reactor each with independent temperature and reaction gas controls. The first micro-reactor vaporizes liquid samples, or pyrolyzes solid samples. Vapors formed in the first reactor flow through the catalyst in the second reactor. The Tandem Micro-Reactor is designed to allow a quick change of the catalyst bed. Adding a cryo-trap and a selective sampler extend the capabilities of the system to include heart-cutting experiments. Batch or continuous experiments can be performed with this system to evaluate both catalyst performance and to characterize products formed on the catalyst surface. Provisions are also designed into the system to allow catalyst regeneration and evaluation. The catalytic pyrolysis of lignin, cellulose and Jatropha “press cake” will be used to illustrate the utility of the Tandem Micro-Reactor. The analysis of lignin in Hydrogen (at high pressure) using Heart-cut EGA-MS analysis will also be discussed.

Keywords: Biofuels, Materials Characterization, Polymers & Plastics, Pyrolysis
Application Code: Polymers and Plastics
Methodology Code: Gas Chromatography/Mass Spectrometry
The global helium shortage and price increase often force analytical laboratories running multiple gas chromatographs to reconsider their helium consumptions. Migrating to another carrier gas like hydrogen is probably the most common choice, even if its adoption brings to some obvious safety concerns.

Despite hydrogen’s well known behavior of allowing the best separation per unit time, thus allowing laboratories to increase their throughput, some analytical considerations must be done as well, which can affect both the injection port as well as the detection technique used.

An innovative solution will be presented in this paper, consisting on the use of a dedicated GC injector, which allows the high separation efficiency of hydrogen carrier gas without incurring into its the negative aspects.

Drastically reducing the amount of hydrogen carrier used, but still allowing its excellent chromatographic separation and rapid GC or GCMS method implementations, this injector provides hydrogen-using gas chromatograph systems with an increased margin of safety over that provided with prior art hydrogen equipped chromatographs. This allows in principle GCs to be using hydrogen carrier gas without the need of hydrogen sensors installed.

Data will be presented to discuss the impact of such an injector on reducing the total amount of hydrogen used and the chemical reactivity sometimes observed towards unsaturated analytes; we will also demonstrate that the adoption of this innovative injector extends the applicability of thermal conductivity detectors, whose sensitivity and performance are known to be affected by carrier gas switching during the run.

**Keywords**: Gas Chromatography, Gas Chromatography/Mass Spectrometry, Instrumentation

**Application Code**: High-Throughput Chemical Analysis

**Methodology Code**: Gas Chromatography/Mass Spectrometry
Fast pyrolysis is one of the techniques being used to convert biomass to biofuels or bio-based intermediates. However, initial work with fast pyrolysis underscored the difficulty of converting biomass to transportation fuels in a single step. Catalytic pyrolysis has emerged as a means for improving the quality of the bio-oil produced from pyrolysis. Catalytic pyrolysis is often done in Hydrogen, usually at elevated pressure. Most of the high pressure, catalytic pyrolysis studies are conducted in room-size reactor systems, which often limits the scope of the study. The introduction of a table-top, high pressure Tandem Micro-Reactor system overcomes this limitation.

A tandem reactor consists of two reactors in series; additional capability can be added via a series of accessories. The atmosphere and temperature (up to 900°C) of each reactor are independently controlled. The system is designed to be operated up to 3.5 MPa (500 psi). Reactor pressure is controlled using a unique back pressure regulator, which has the advantage of being downstream of the reactors. Only the portion of the sample being split flows through the regulator. A small portion of the sample vapors flows directly to the column. A second back pressure regulator maintains a constant pressure on the column, so that retention data is constant and independent of the reactor pressure.

Heart-cut EGA GC/MS analysis of lignin under high pressure in a hydrogen atmosphere will illustrate how the Tandem Micro-Reactor GC/MS is used to investigate the potential of various feedstocks and novel catalyst. The products formed after pyrolysis and catalytic conversion of cellulose will be used to highlight the various operating modes of the tandem reactor.

Keywords: Biofuels, Instrumentation, Polymers & Plastics, Pyrolysis
Application Code: Polymers and Plastics
Methodology Code: Gas Chromatography/Mass Spectrometry
Sample identification by GC-MS is typically performed with library search among available electron ionization (EI) mass spectra via the availability of fragments, automatically providing compound names and identification probabilities. Significantly improved methods of sample identification were explored using the Aviv Analytical 5975-SMB GC-MS with Cold EI, an instrument based on the use of supersonic molecular beams (SMB) for interfacing the GC with MS and EI of vibrationally cold sample compounds in SMB with a fly-through ion source. The following sample identification aspects were improved:

A) The use of short columns with high column flow rates enables significant extension of the range of compounds amenable for GC-MS analysis, while the fly-through ion source ensure their ionization without ion source degradation, thereby bridging the gap with LC-MS.

B) Enhanced molecular ions significantly improve library search and confidence in identification.

C) The combined availability of enhanced molecular ions and Tal-Aviv Molecule Identifier (TAMI) software based on isotope abundance analysis serves to confirm or reject NIST results.

D) The TAMI software further provides elemental formula based on the combination of isotope abundances and increased mass accuracy measurement of the molecular ions masses with single quad MS.

E) The combination of enhanced molecular ions and high mass fragments provides extended structural and isomer information.

GC-MS with Cold EI and TAMI software is superior to GC-HR-TOF in sample identification since greater range of compounds can be eluted and exhibit trustworthy molecular ion with it, while the TAMI software provides elemental formula.

Keywords: Gas Chromatography/Mass Spectrometry, GC-MS, Identification, Software

Application Code: Other

Methodology Code: Gas Chromatography/Mass Spectrometry
Acquisition of quality mass spectra of analytical derivatives for important chemicals is essential for the improvement of the NIST/NIH/EPA Mass Spectral Library. Thermally and catalytically liable compounds, compounds with poor gas chromatography properties, and compounds containing insufficient characteristic ions and structural information in their mass spectra are on the top of our priority list. The presentation will describe derivatization methods used in the GC/MS leading to (a) locating centers of unsaturation, branching and positions of functional groups, and (b) determining cycle ring size and positions of substituents. Advantages and disadvantages of a particular derivative for a specific compound will be assessed. Special attention will be paid to procedures for preparation of chemical derivatization products with two or more different functional groups; recommendations for their synthesis will be given. The fragmentation pathways of compounds of interest and their derivatives, as well as characteristic ions in their mass spectra will be discussed. Furthermore, evaluation methods of newly acquired mass spectral data of analytical derivatives for the addition to the NIST/NIH/EPA mass spectral library will be described.

Keywords: Database, Derivatization, Gas Chromatography/Mass Spectrometry, Quality Control
Application Code: General Interest
Methodology Code: Gas Chromatography/Mass Spectrometry
Methods for Cancer Detection

Isolation of Low Abundance Cancer Cells from Blood Using Surface Acoustic Waves

Circulating tumor cells (CTCs) open up the opportunity to examine and study cancer progress with a more non-invasive manner (blood drawing). However, to better understand CTCs and its relation with disease progression still calls for new methods that are able to purify the extremely rare CTCs from blood sample. In recent years, separation of cells using acoustic forces has drawn increasingly amount of attentions due to the advantages of flexibility, contact-less, and high bio-compatibility. However, isolating CTCs from blood sample has not been possible using acoustic based methods due to the insufficient throughput or separation stability. In this work, we increase the separation distance and throughput between cancer cells and leukocytes by optimizing the configuration of standing wave field in a microfluidic channel. As a result, isolation of CTCs from blood with reasonable throughput and efficiency becomes possible. The device achieved >80% separation efficiency with as few as 60 cells/mL. The current single device is able to process 1.2 mL of blood per hour, and cells are still viable after separation and can be cultured for one week.

Keywords: Bioanalytical, Biomedical, Isolation/Purification, Lab-on-a-Chip/Microfluidics

Application Code: Biomedical

Methodology Code: Microfluidics/Lab-on-a-Chip
Multiplexed protein detection methods for molecular diagnostics have struggled to enter into a clinical setting in part due to the lack of convenient analytical tools. Microring resonator arrays are a class of silicon photonic biosensors that are well suited for multiplexed protein measurements in complex matrices following a similar workflow to conventional sandwich immunoassay methods. The platform consists of individually addressable rings arrayed across a silicon surface with current generation chips capable of up to 32-plex analysis. The assay is highly automated with integrated, programmable fluidic control with rapid data acquisition (<2 h) and picomolar limits of detection. Herein, we demonstrate the application of the microring resonator platform for simultaneous analysis of 12 phosphoprotein targets in model cancer cell lines and tumor tissue homogenate. The assay is able to discriminate heterogeneities between model cell lines as well as monitor alterations in the proteomic profiles in response to environmental changes. Applications of the phosphoprotein expression profiling include monitoring disease progression, detection of emerging resistance mechanisms, correlating protein modifications with oncogenes, and additional categorization of cancers into clinically actionable sub-groups.

Keywords: Array Detectors, Biotechnology, Immunoassay, Protein
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Gliomas are the most frequent primary brain tumours in adults, with these intracranial neoplasms accounting for 70% of adult malignant brain tumours [1]. The current diagnostic regime is subjective, invasive, and may require the patient to undergo unnecessary surgery.

Studies have shown the potential of ATR-FTIR spectroscopy for diagnostic applications when collecting spectral measurements of human serum to discriminate between healthy and diseased states; studies involve cardiology [2], ovarian cancer [3-4] and our previous studies on brain cancer [5-6], to name but a few.

We used ATR-FTIR spectroscopy combined with a Radial Based Function Kernel Support Vector Machine (RBF-SVM) to diagnose high-grade gliomas (e.g. Glioblastoma multiforme), low-grade gliomas (e.g. oligoendroglialoma) and non-cancerous samples using serum volumes of 1 µl. Optimum sensitivities and specificities of 93.75 and 96.53% respectively, were achieved when analysing whole serum samples. We built upon our existing spectral dataset to construct 5 models; (1) cancer vs. non-cancer, (2) metastatic brain cancer vs. brain cancer vs. non-cancer, (3) organ of tumour origin, (4) high grade glioma vs. low grade glioma vs. meningioma, (5) subtype of brain cancer. In total our latest research includes 433 patients demonstrating a rapid stratified serum diagnostic process using minimal sample.

We present a rapid, robust and reproducible methodology that can be used to diagnose and distinguish between cancer, organ origin and tumour severity from non-cancer with a 1 µl volume of human serum with high sensitivity and specificity.

References

Keywords: Bioanalytical, Biomedical, Biospectroscopy, Infrared and Raman
Application Code: Biomedical
Methodology Code: Biospectroscopy
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**Session Title**: Methods for Cancer Detection  
**Abstract Title**: Paper Chemiluminescence ImmunoPAD: Rapid Detection of Cancer Biomarker Proteins Using Magnetic Bead Bioconjugates  
**Primary Author**: Chi Tang, University of Connecticut  
**Co-Author(s)**: Abhay Vaze, James F. Rusling  
**Date**: Monday, March 09, 2015 - Afternoon  
**Time**: 03:25 PM  
**Room**: 271  
**Bioanalytical, Chemiluminescence, Immunoassay, Lab-on-a-Chip/Microfluidics**  
**Biomedical**  
**Microfluidics/Lab-on-a-Chip**

Abstract Text

Development of technically simple, inexpensive devices for cancer detection has the potential to enable early detection and personalized medicine. Paper has attracted interest as a cheap, versatile material for development of clinical diagnostic measurement tools for analytes in urine and blood. Paper analytical devices (PADs) have the potential for rapid testing of biological samples to offer real time disease diagnostic tests to assist physicians in delivering personalized treatment and improve prognoses for patients. A low cost, high throughput and disposable paper analytical pad with 64 analytical wells was fabricated using wax patterned filter paper. The PAD interfaced with capture antibodies decorated magnetic microbeads and chemiluminescence reagents was used to detect cancer biomarker proteins prostate specific antigen (PSA) in undiluted serum at pg mL\(^{-1}\) levels. Capture antibodies for PSA was first immobilized onto magnetic beads and a sandwich type immunoassay involving antigen (standards or patient samples), biotinylated antibodies, and streptavidin-polyHRP was carried out. The chemiluminescence output was measured with a charge-coupled device (CCD) camera and total assay time from antigen immobilization to qualitative results were completed in ~20 mins for all 64 analytical spot. Detection limits of 2 pg mL\(^{-1}\) for PSA were obtained in undiluted serum and a clinical relevant dynamic range of 4 logarithmic orders was achieved. These disposable low cost PAD presents an ideal candidate for rapid, high throughput diagnostic tool for serum proteins.

**Keywords**: Bioanalytical, Chemiluminescence, Immunoassay, Lab-on-a-Chip/Microfluidics

**Application Code**: Biomedical

**Methodology Code**: Microfluidics/Lab-on-a-Chip
DNA nanotechnology has explored its universal application by constructing various functional nanostructures. As a rising star applied in biomedical field, DNA nanostructures have been used as anticancer drug carriers to decrease the systemic toxicity resulting from nonspecific drug distribution and enhance drug delivery efficiency. Here, we report an aptamer-based DNA dendritic nanostructure as a multifunctional vehicle for targeted cancer cell imaging and drug delivery. The multifunctional DNA dendrimer is constructed by functional Y-shaped building blocks with predesigned base pairing hybridization including fluorophores, targeting DNA aptamers and intercalated anticancer drugs. With the controllable step-by-step self-assembly, this programmable DNA dendrimer has appealing features such as facile modular design, excellent bio-stability, good biocompatibility, high selectivity, strong binding affinity, efficient cell internalization property, and large drug loading capacity. Due to the unique structural features of DNA dendrimers, multiple copies of aptamers can be conjugated in each one dendrimer, generating a multivalent aptamer-tethered nanostructure with enhanced binding affinity. The high content of drug-binding double-strand DNA structures in these dendrimers enabled them with high drug payload capacity. A model chemotherapeutic anticancer drug, doxorubicin (Dox), was delivered via these aptamer-based DNA dendrimers and exerted a potent toxicity for target cancer cell with low side effects for the non-target cells. Therefore, this controllable aptamer-based DNA dendrimer presents a promising candidate for biomedical applications.

Acknowledgement: We thank the National Basic Research Program of China (2010CB732402, 2013CB933703), the National Science Foundation of China (21205100, 21275122, 21075104), National Instrumentation Program (2011YQ03012412) for their financial support.
Circulating Tumor Cell Analysis for Cancer Metastasis Using a Novel Track-Etched Magnetic Micropore (TEMPO) Filter based Microfluidic Device

Pancreatic cancer is highly lethal cancers, with a 5% 5-year survival rate [1,2]. In recent work by our collaborator, it has been shown that at the time of diagnosis most patients are metastatic [1, 3-6]. Thus, there is both a great need for early diagnosis and an opportunity, due to the early presence of circulating tumor cells (CTCs) [7, 8]. The detection of CTCs from blood poses a technical problem, due to their extreme scarcity (1 in 10E9 blood cells) and the lack of any biomarker that can be used to identify 100% of the CTCs. Microfluidic approaches have been used to isolate CTCs, but limited by biased sorting (missing non-epithelial CTCs), reliance on downstream analysis that can cause cell loss and impractically slow throughput for clinical application [9].

To address these issues, we developed a platform that can sort CTCs from the vast background of blood cells, and screen each cell individually using on chip in-situ RNA analysis. We sort white blood cells using negative immunomagnetic selection. Our chip uses a novel track-etched magnetic micropore (TEMPO) filter based microfluidic geometry. By using magnetic micropores, magnetically labeled blood cells can be removed directly from unprocessed whole blood at high flow rates (≤ 10 mL/hr). The TEMPO consists of a thick (1.25 micron) electroplated NiFe layer (Fig 1a), 30 micron diameter pores with density of 4x10E5 pores/cm2, creating strong magnetopheric forces while minimizing flow velocity and clogging. To sort the rare CTCs, we demonstrated extremely low loss (Fig 1b) and exponentially improved sorting efficiency by stacking TEMPO filters in series (Fig 1c). Downstream of the TEMPO, potential tumor cells are trapped on a size-based filter for fluorescence in situ hybridization (FISH) that could resolve a single molecule of RNA in individual cells (Fig 1d), with the specificity to resolve a single point mutation. We are applying this technique to clinical and murine mouse models for pancreatic cancer.

Keywords: Biomedical, Detection, Isolation/Purification, Lab-on-a-Chip/Microfluidics
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Online analyte preconcentration is a preferred method to increase the limited detection sensitivity of miniaturized capillary electrophoresis (CE) resulting from its small sample injection volume. We have developed a microfluidic platform that employs a pneumatic microvalve for controlled sample injection for CE. A pneumatic microvalve, created using multilayer soft lithography, aligned at a T-junction on PDMS chip can serve as a semipermeable membrane under an applied potential, enabling current to pass through while blocking the passage of charged analytes. For sample preconcentration, analytes are focused at the closed valve. Once analytes are concentrated, the valve is briefly opened and the stacked sample is injected onto an integrated microchip or capillary column. Preconcentration and injection of cationic and anionic species has been demonstrated using crystal violet and fluorescein, respectively. Fluorescein was enriched by a factor of ~70 to 190 µM in just 8 s as determined using a calibration curve and the concentrated analyte was injected as a 1 nL plug onto the CE channel. Compared to existing preconcentration approaches, our method enables both rapid analyte concentration and controlled injection volume for high sensitivity and high resolution CE. Additionally, this may present a promising method for coupling two-dimensional separations, such as liquid chromatography coupled to CE.

Keywords: Capillary Electrophoresis, Lab-on-a-Chip/Microfluidics, Sample Introduction, Sampling
Application Code: High-Throughput Chemical Analysis
Methodology Code: Microfluidics/Lab-on-a-Chip
Improved monitoring of cellular systems can be achieved with microfluidics. Flow over cells on-chip provides a continuous source of nutrients and allows real-time sampling of secreted metabolites. Using lab-on-chip techniques, we have integrated a dual enzyme assays system on a chip with a cell perfusion chamber to create an automated, multiplexed, and miniaturized platform for cellular monitoring. Adipocytes secrete non-esterified fatty acids (NEFAs) and glycerol through a metabolic pathway known as lipolysis. Measuring both products allows assessment of metabolic function and NEFA recycling, which refers to re-esterification of NEFAs after lipolysis within a cell. Measuring secretion can provide insight into the physiological conditions of the cells. For example, an imbalance of lipolysis and NEFA recycling is often associated with type 2 diabetes and various other disorders. A novel, multilayer polydimethylsiloxane (PDMS) chip has been developed to detect both NEFAs and glycerol secreted from a group of adipocytes in near real time to better understand NEFA recycling. The reversibly-sealed chip integrated a valve, cell chamber and parallel NEFA and glycerol enzyme assay reaction channels. Using the system, 3T3-L1 adipocytes were continuously perfused in the chamber, and the on-chip assays were optimized to allow detection of the secreted metabolites and changes in recycling as a function of age and drug treatments. To provide more detail on the chemical identity of NEFAs that are secreted, we have also coupled the chip to solid-phase extraction with ESI-MS for lower temporal resolution monitoring. This system provides simple coupling of microfluidics to MS for cellular assessment.

**Keywords:** Enzyme Assays, Lab-on-a-Chip/Microfluidics, Mass Spectrometry, On-line
**Application Code:** Bioanalytical
**Methodology Code:** Microfluidics/Lab-on-a-Chip
Recent advances in proteomics are facilitating the study of increasingly complex systems. However, traditional one-dimensional separation methods are capable of analyzing only a few dozen analytes, which is nowhere near the thousands of components typically present in a biological sample. Multi-dimensional separations offer an advantage since separations can be done based on multiple properties. Much interest has been placed in the field of comprehensive on-line two-dimensional separations, where two columns which provide orthogonal separation methods are directly connected. One of the main limitations to this technique is that the rate at which the first dimension is sampled is dependent upon the time it takes to fully run and re-equilibrate the second dimension. For the first time, micro free-flow electrophoresis (µFFE) has been directly coupled with nano-liquid chromatography (nLC) for amino acid and peptide separations. µFFE is a continuous separation technique where samples are introduced to the top of a separation chamber and are driven by pressure flow. The flow rate and capillary size for samples are comparable to that found in nLC, making it ideal for coupling. An electric field is applied laterally, and the analyte streams begin to move and separate according to their electrophoretic mobilities. Because the separation is continuous, the sampling rate becomes dependent only on the time required for detection, typically 100 msec. The research presented demonstrates the orthogonality and separation capabilities of nLC- µFFE by separating a 25 amino acid mixture and tryptic peptides of BSA using reversed-phase LC and various µFFE conditions. By implementing a near zero dead volume interface between the nLC instrument and µFFE microfluidic device peak capacities of over 2300 have been achieved in a 10 minute separation window for peptides, and near 100 for amino acids in 75 seconds.

This work was supported by NSF-CHE Grant #1152022.

Keywords: Capillary LC, Electrophoresis, Lab-on-a-Chip/Microfluidics, Protein
Application Code: Genomics, Proteomics and Other 'Oomics
Methodology Code: Microfluidics/Lab-on-a-Chip
It is well established in drug discovery that in vitro tools capable of dynamic in vivo processes are lacking. Conventional polydimethylsiloxane (PDMS) based thrombosis models, while able to recreate in vivo dimensions, suffer from a lengthy fabrication process, final devices are often not reusable, and to date fail to enable localized injury to an endothelium triggering the thrombosis pathway. A polyjet 3D-printer is used to fabricate a reusable, transparent fluidic device featuring threaded inlets for sample and removable electrode introduction, and a 1000 µm wide x 800 µm high channel. Channels are coated with either PDMS or polystyrene (PS), well characterized substrates that are amenable to cell adhesion and growth. These coatings decrease the internal channel dimensions, 3-100 µm (PDMS) and >100 µm (PS) as confirmed with scanning electron microscopy, but have limited effect on device transparency. Collagen encompassing the internal channel provides the extracellular matrix component and aids in endothelial cell immobilization. Injury to the endothelium is performed in a site-specific manner by electrically lysing cells via potential application to electrodes inserted into the base of the device, providing the basis for thrombosis formation. Electrical lysis facilitates injury without introducing any foreign circulation components, expediting the process by foregoing the need to prep the channel before sample introduction. Whole blood is introduced to the device after electrically-induced injury to form the blood clot, which can be imaged using microscopy techniques or used to assess anti-thrombotic or thrombolytic drugs.

Keywords: Biotechnology, Electrodes, Lab-on-a-Chip/Microfluidics, Pharmaceutical
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
High-throughput screening (HTS) using multiwell plates and fluorescence assays is a powerful tool for drug discovery by allowing tens of thousands of assays to be completed per day. While this method has been successful, typically only one analyte is detected and indicator reactions can lead to false results. We have developed a novel method using droplet microfluidics to couple multiwell plate-based assays to microchip electrophoresis (MCE) for screening. Samples contained in multiwell plates are reformatted in to nanoliter volume plugs segmented by an immiscible oil. The segmented flow sample streams are coupled to a hybrid polydimethylsiloxane-glass microfluidic device capable of selectively extracting the aqueous samples from the droplet stream and rapidly analyzing by MCE with laser-induced fluorescence detection.

To validate this new method, a test library containing 140 small molecules were screened for inhibitors of protein kinase A (PKA). Each sample was reformatted as two droplets allowing at least 6 MCE injections per sample. Sub-second separation of three analytes – rhodamine, substrate peptide, and product peptide – was achieved in 5 mm using an applied electric field of 2000 V/cm with an efficiency of 16,000 plates/second. Using 700 injections total, 96 samples were analyzed in 12 minutes (0.13 Hz) and several small molecule inhibitors of PKA were identified and verified through dose-response experiments. While this method has been applied to screening applications, the device layout and operation is amenable to many applications including coupling 2D separations and chemical sensing. This work was supported by funding from the National Institutes of Health.
In recent years, microfluidic paper-based analytical devices (PADS) have drawn much interest as platforms for analysis in a variety of complex biological and environmental systems. PADS have many benefits due to the unique properties of paper including capillary action, reagent compatibility, widespread availability, and low cost. Common fabrication methods include photolithography, wax printing/dipping, and inkjet printing; all suffer drawbacks including high cost, difficult post processing, unstable hydrophobic agents, limited applications, and non-biodegradability.

Polycaprolactone (PCL) is a biodegradable polyester with a melting point of 60°C that can address many of the current issues. PCL is commonly used for manufacturing specialty polyurethanes and for coating extended release tablets. In the present study, PCL is used as both a hydrophobic barrier and adhesive agent in the fabrication of single, and multilayer microfluidic devices. PADS were prepared by airbrushing PCL solutions onto masked substrates. Hybrid microfluidic devices comprising both open channels and paper wicking regions were prepared by impregnating filter paper with PCL solutions followed by drying, cutting, and assembly. In both methods, CAD software was used to generate designs, which were then transferred to prepared substrates with a cutting plotter. Multilayer devices were then laminated to create three-dimensional structures.

We employed this fabrication process to produce colorimetric diagnostic assays for clinically relevant biomarkers including glucose, albumin, bilirubin, and creatinine. Assay results were initially analyzed off-line using commercially available software. In order to maximize portability, a smartphone application was developed allowing for quantitative and statistical analysis of colorimetric assays without the need for a computer.

Keywords: Clinical Chemistry, Lab-on-a-Chip/Microfluidics, Polymers & Plastics, Software

Application Code: Bioanalytical

Methodology Code: Microfluidics/Lab-on-a-Chip
Deficiencies on essential metal ions such as copper, zinc and iron could lead to various diseases that are detrimental to human health. Therefore, it is important to quantitatively monitor the concentrations of these metal ions in a simple, cost-effective, point-of-care and reliable fashion. Here, we present a fully inkjet-printed microfluidic paper-based analytical device (\(\text{[micro]}\)PAD) with pre-treatment and ion sensing functions on a filter paper material for the simultaneous color image analysis of \(\text{Cu}^{2+}\), \(\text{Zn}^{2+}\) and \(\text{Fe}^{2+}\) ions. Microliter order of sample containing metal ions was dropped on a single sample compartment that instantaneously flowed into the 3 different pre-treatment area then into the ion sensor. The printed pre-treatment reagents on the \([\text{micro}]\)PAD were mainly composed of either buffers with specific pH or a mixture of reductant and masking agent, which aided for more selective measurements. As for the ion sensing, Zincon dye was used to measure both the \(\text{Cu}^{2+}\) and \(\text{Zn}^{2+}\) ions and Ferene S dye for the \(\text{Fe}^{2+}\) ion. These dyes were printed on cellulose-anchored cationic polymer nanoparticles (~100nm) via electrostatic interaction that resulted to a more reliable measurement. The normal serum level of each cations were well within the calibration standard obtained by the \([\text{micro}]\)PAD. Moreover, simultaneous analysis of each metal ions were successfully demonstrated. This simple and cost-effective fully inkjet-printed \([\text{micro}]\)PAD could be implemented for clinic and field applications.

**Keywords:** Lab-on-a-Chip/Microfluidics, Sensors

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Point-of-care testing (POCT) with the advantages of speed, simplicity, and low cost, as well as no need for instrumentation is of great importance to improve healthcare, ensure environmental safety, and guarantee food quality, especially in a variety of environments that lack access to laboratory infrastructure, such as in less-industrialized countries, in emergency situations, or in home healthcare settings. While qualitative POC assays are widely available, quantitative POC assays post significant challenges. Here we describe a novel method that integrates an Au core/Pt shell nanoparticle (Au@PtNP) encapsulated aptamer-crosslinked hydrogel with a volumetric bar-chart chip readout (HV-Chip) for sensitive, selective, and quantitative POCT with naked eyes. Upon target introduction, the formation of target/aptamer complex destroys the hydrogel crosslinks, leading to the dissolution of the hydrogel to release Au@PtNPs. The resulting nanoparticles can efficiently catalyze the decomposition of H2O2 to generate a large volume of O2 to push the movement of an ink bar in the V-Chip. The concentration of target introduced can be visually quantified by reading the travel distance of the ink bar. By changing the aptamer sequences, the HV-Chip method allows for inexpensive, rapid, portable, and quantitative visual detection of a wide range of targets without any external electronic devices, which has the great potential to be used for quantitative POCT by local communities in developing regions to improve healthcare, environmental safety, food quality, etc.

Acknowledgement: We thank the National Basic Research Program of China (2010CB732402, 2013CB933703), the National Science Foundation of China (21205100, 21275122, 21075104), National Instrumentation Program (2011YQ03012412) and the Natural Science Foundation of Fujian Province for Distinguished Young Scholars (2010J06004) for their financial support.

Keywords: Biosensors, Lab-on-a-Chip/Microfluidics, Nucleic Acids, Portable Instruments
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Ion chromatography (IC) based methods are appearing in greater number in the United States Pharmacopeia National Formulary (USP-NF). In addition to the counter ion analysis assays commonly performed at pharmaceutical companies, there are now IC assays of drug substances and drug products, and methods to determine related substances and impurities in drug substances and drug products. Most IC assays feature an ion-exchange separation followed by suppressed conductivity, pulsed electrochemical, or UV absorbance detection. This presentation will describe four recently developed IC assays for active pharmaceutical ingredients (APIs) as well as impurities. Some of these methods are part of an ongoing effort of monograph modernization (e.g. the determination of ammonia in bicarbonate). Lithium carbonate will be used as an example of an API assay as well as for an assay of sodium and calcium impurities. In this example one IC method replaces three separate methods in the monograph. The other two application examples will feature anion-exchange IC. The discussion of all four application examples will emphasize the column, mobile phase, and detection choices made during the development of each method.
Analytical characterization of monoclonal antibodies is required during the manufacturing process and clinical studies. Structural heterogeneity such as intra-molecular disulfide scrambling can be induced during all aspects of the manufacturing process. For IgG2, disulfide scrambling is especially a problem due to the four disulfide bonds in the hinge region. RPLC has been used to partially resolve four peaks for IgG2, corresponding to three disulfide isomers. It is unclear about the cause of the extra peak. In this study, SEC results prove that aggregates can be induced from the mobile phase used in RPLC. The percentage of aggregates formed is related to sample concentration. Only the separation at low concentration can reflect the real composition of disulfide isomers in the original sample. The separation efficiency can be improved by using submicron nonporous particles. The higher separation efficiency, together with optimized bonded stationary phase allows for higher resolution separation of IgG2 disulfide isomers.

Keywords: Biopharmaceutical, HPLC, HPLC Columns, Liquid Chromatography/Mass Spectroscopy
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
When core-type particles are available with the desired phase chemistry, there are compelling reasons to include them during HPLC and UHPLC method development. Because core-type particles typically generate more theoretical plates than porous particles for a given diameter, one option is to choose the same core-type particle size and generate higher efficiency and resolution at the same operating pressure. Another option is to select a larger core-type particle size and achieve equivalent efficiency and resolution at lower pressure. This efficiency/pressure advantage can be an attractive tool when methods may be used by multiple analysts on instruments with different performance capabilities. The core-type efficiency advantage typically increases at high flow velocities, which is critically important for any method that has greater separation speed as an objective. A second important advantage for core-type columns is that they have been reported to be more rugged in use compared to porous columns, especially with smaller particles inside. This makes them ideal for unattended runs during column switching and screening or for multi-sample assays where loss of column performance during the run can be very costly. The efficiency/pressure and ruggedness advantages become even more important in UHPLC methods because operating pressures may become limiting and column performance losses at high pressure may decrease resolution and accuracy. Because core-type and porous particles have similar retention factors, they may be screened together to gain experience with both column types. Since their introduction in 2007, core-type particles have become widely available for HPLC and UHPLC with a variety of column geometries and phases that should make development of rugged methods faster and easier.
The use of superficially porous particles (SPP) and sub 2-μm fully porous particles (FPP) in achiral applications has rapidly grown in the past decade and has allowed for extremely fast and efficient achiral separations. However, chiral separations have yet to follow the trend. Chiral separation are notorious for low efficiency due to required strong interaction between the analytes and the stationary phase. In this work, HPLC columns packed with bonded, brush-type chiral selector were produced using 2.7-μm SPPs and 1.9-μm mono-dispersed FPPs. These new, highly efficient chiral stationary phases are used to rapidly separate a wide range of chiral analytes in less than 60 seconds. These high efficiency columns can be used for fast separation of chiral analytes in pharmaceuticals for increased throughput and for use in multidimensional (2-D) separations. Short columns packed with these particles can also be used for rapid screening of large number of analytes in a very short time frame.
Degradation products such as aggregates and fragments pose a critical problem in monoclonal antibody (mAb) drug development. Reduced therapeutic efficacy and immunogenic responses in patients have been associated with these drug impurities. Conventional reversed phase stationary phases such as porous and core shell packing can currently separate intact mAb fragments but are still limited in the separation of aggregates. Capillaries packed with submicron nonporous silica particles have been used to successfully separate mAb aggregates using reversed-phase chromatography with baseline resolution at room temperature. Since stainless steel RPLC columns are more widely used as an analytical tool for mAb separations in the pharmaceutical industry, it is desirable to translate this efficient separation in capillaries to a form that is amenable with commercial UHPLC instrumentation. This work examines the progress being made in using nonporous submicron silica particles packed in stainless steel columns for both mAb fragments and aggregate characterization. Preliminary results show that stainless steel columns packed with submicron nonporous silica particles separated mAb fragments and aggregates simultaneously from its monomer with better resolution than leading commercial RPLC columns.

This work is supported by grant NIH R01 GM1011464.

Keywords:  
HPLC, HPLC Columns, Liquid Chromatography, Pharmaceutical

Application Code:  
Pharmaceutical

Methodology Code:  
Liquid Chromatography
### Abstract Text

The separation of chiral compounds continues to be the center of significant interest not only in the development of pharmaceutical drugs and therapeutics but also in agricultural industry (herbicides and pesticides) as well as biomarkers.

Capillary electrophoresis (CE) is a highly effective technique for chiral separation, which is superior to gas chromatography (GC) for analyzing non-volatile chiral compounds and has some advantages over high performance liquid chromatography (HPLC) in terms of the cost, sample consumption and etc.

The combination of CE to mass spectrometry (MS), a hyphenated technique, largely improves the limit of detection of CE to analyze both small and large molecules by mass difference. However, most of the small molecular weight chiral selectors conventionally used in CE suppress the MS detection to a large extent. Therefore, identifying novel chiral selectors which provide high separation selectivity and are MS compatible has become a major effort in developing CE-MS approaches.

According to the literature, sugar based surfactants unpolymerized micelles have low critical micelle concentrations of about 0.5 mM and have been successfully used as pseudostationary in MEKC for the separation of enantiomers. Based on this report, a new series of molecular micelles, the sugar based surfactants with vinyl terminated group have been synthesized and been used to separate some ephedrine alkaloids, β-blockers, and binaphthyl derivatives in MEKC-MS.

### Keywords

- Analysis
- Chiral Separations
- Chromatography
- Mass Spectrometry
Efficient peptide separation is essential for proteomic research and therapeutic peptide development in the pharmaceutical industry. By using submicron nonporous particles, higher efficiency can be achieved due to better-ordered stationary phase, reduced mass transfer and slip flow enhancement. In this work, 500 nm silica particles were packed into a 150 μm i.d. capillary to form C18 bonded stationary phase, with a pulled tip to couple to mass spectrometer. Using bovine serum albumin tryptic digest as a standard sample, a peak capacity of over 300 was achieved in a 17 min gradient. High sensitivity of peptides was observed in the mass spectrum. Various percentages of formic acid and trifluoroacetic acid in mobile phase were applied to study the influence of different solvent conditions. Human B cell tryptic digest was used to test the ability of proteomics sample separation. In a 10 min gradient, a peak capacity of over 200 was obtained. Thus, our capillary shows great potential for highly efficient peptide separation in proteomics study and pharmaceutical application.

This work is supported by NIH R01-GM101464.

Keywords: Biopharmaceutical, Capillary LC, Liquid Chromatography/Mass Spectroscopy, Proteomics
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Pharmaceutical- LC, LC/MS

Effect of Calliandra Portoricensis on the Pharmacokinetics of Glibenclamide in Rats

Glibenclamide, a second generation sulfonylurea is a diabetic drug which functions by stimulating insulin secretion. Calliandra portoricensis is used in the treatment of snakebite, antiulcer effects and as abortifacient. The pharmacokinetic interaction of glibenclamide and calliandra portoricensis (CP) was therefore investigated in rats.

Twenty four Wistar rats of both sexes weighing 150 ± 15g, received a dose of glibenclamide, 0.6mg/kg body weight and two weeks later, they were administered 500mg/kg of calliandra portoricensis single-oral doses for days 1-5 and also received 0.6mg/kg glibenclamide single-oral doses 15 minutes post-administration on the fifth day. Blood samples were withdrawn by retro-orbital puncture using capillary tubes over a 48 h period on both occasions and the quantity of glibenclamide in the plasma at each time was determined by HPLC.

The observed decrease in the elimination half-life, t½ and maximum plasma concentration, Cmax, as well as increase in time to reach maximum plasma concentration, T max and volume of distribution, Vd were not significant when glibenclamide was co administered with calliandra portoricensis. The lower Cmax and longer Tmax observed could be an indication of reduced rate of drug absorption. This was shown by the reduction in AUC. Drug elimination was slower on administration of ciprofloxacin with calliandra portoricensis as there was a decrease in the elimination half-life, t½ and increase in volume of distribution, Vd. These findings suggested a drug – drug interaction between ciprofloxacin and calliandra portoricensis. Thus, the implication of the concomitant administration of these two should not be overlooked.

Keywords: Biological Samples, Biopharmaceutical, HPLC, Liquid Chromatography
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Selective extraction, solvent fractionation, subsequent chromatographic separation are eliminated in favor of total lipid extraction followed by tandem mass spectrometric analysis. The total lipid extract is applied directly via electrospray ionization, followed by neutral loss scanning to enable ion selection for lipids in the desired mass/charge range based on the offset of either one (monogalactosyldiglycerides) or two (digalactosyldiglycerides) galactose groups after fragmentation. This reveals the acyl group population that appears for the glycolipid and subsequent quantitation.

Keywords: Biological Samples, Lipids, Quantitative, Tandem Mass Spec
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Dried blood spot (DBS) analysis is becoming the avenue of choice for many pharmaceutical, toxicological, and forensic studies. Aside from economic benefits of shipping DBS cards rather than vials of blood, this method allows for smaller volumes of blood to be collected, which is advantageous in pediatric and geriatric studies. The traditional method of analyzing DBS is liquid chromatography-tandem mass spectrometry (LC-MS\textsuperscript{n}), which requires extracting the blood and any analytes from the paper substrate. This process is time-consuming, difficult to automate, and can dilute the sample. For these reasons, direct analysis techniques have become of interest, as they offer the potential for more rapid analysis. Paper spray ionization tandem mass spectrometry (PS-MS\textsuperscript{n}) by 3-D ion trap and matrix-assisted laser desorption/ionization tandem mass spectrometry (MALDI-MS\textsuperscript{n}) by linear ion trap are compared in this research for application to DBS analysis.

In addition, quantification of compounds in blood has been a focus of many studies. However, quantification by MALDI-MS is challenging due to non-homogenous crystal formation and laser shot-to-shot variations, which lead to irreproducible signal intensities. To overcome these challenges, an internal standard (IS) is added to help improve signal stability. Wide-isolation MALDI-MS\textsuperscript{n}, in which the analyte and internal standard signals are analyzed in a single isolation window, reduces the singal variability and allows for quantification. Quantification by additional direct analysis methods have also been explored.

**Keywords:** Bioanalytical, Mass Spectrometry, Quantitative, Toxicology

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
The reduction in column diameter from traditional 2.1 mm i.d. to column i.d. of < 300 um has been historically employed due to its attribute of delivering greater sensitivity with a lower sample volume requirement when coupled with mass spectrometry. However, in the 20 years that these types of separations have been made widely available, they have predominantly been applied to proteomics analysis in conjunction with high resolution mass spectrometry (HRMS). In contrast to the fused silica columns that are typically utilized for these analysis, integrated microfluidic devices (MFD) offer the ability to operate at elevated pressures, increased resolution, and in a more robust manner.

In the work presented here we show data for a variety of biofluid samples analyzed with the incorporation of MFD in conjunction with HRMS. Robustness testing utilizing samples prepared from plasma, urine, bile and microsomal incubations were carried out utilizing typical bioanalytical sample preparation techniques. The quantitative data produced with the MFD/HRMS approach produced equivalent s/n values for probe pharmaceutical compounds when compared to analysis under standard analytical UHPLC coupled with tandem quadrupole mass spectrometry. Analytical methods utilizing this approach were capable of obtaining low pg/mL sensitivity with calibration curve linearity of 3 orders of magnitude or better. We also explore the use of the MFD coupled with HRMS to obtain better quality simultaneous Qual/Quan data for bioanalytical studies.
How proteins interact with one another and assemble on a structural basis in addition to the identification of their function(s) is key to understanding biological processes. Native mass spectrometry (MS) has been used successfully to characterize intact protein complexes, providing stoichiometry and structural information that is complementary to conventional structural biology techniques. In this study, native top-down MS/MS approaches, including ISD, CAD, IRMPD, and ECD, using ultra-high resolution Fourier transform ion cyclotron resonance (FT-ICR) MS with nano-electrospray ionization (nano-ESI) were established to characterize the structures of soluble protein assemblies to beyond 800 kDa, and then further extended to a more challenging system — membrane protein complexes in nanodiscs.

The results demonstrate that with the superior high resolving power, accurate mass accuracy, and the versatile fragmentation techniques of FT-ICR MS, rich information, including the molecular weights of the protein complexes (and thereby stoichiometry), amino acid sequence, metal/ligand binding sites, and PTMs can be accomplished in a single native top-down FT-ICR MS experiment for soluble protein complexes. Nanodiscs consist of membrane scaffold proteins that wrap around a hydrophobic core of lipids. Although nanodiscs provide a native-like environment for membrane proteins, the inherent lipid heterogeneity poses a great challenge for MS analysis. With the hyphenation of various fragmentation techniques, not only can membrane protein complexes be released from nanodiscs, but the levels of phospholipid incorporation into the nanodiscs can be revealed. Therefore, native top-down MS/MS can be used to gain structural insights of protein complex topologies.

Support from the US National Institutes of Health (R01GM103479 and S10RR028893 to J.A.L.), the US Department of Energy, and the American Society for Mass Spectrometry Postdoctoral Award Program (to H. L.) are acknowledged.

Keywords: Mass Spectrometry, Protein, Tandem Mass Spec
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Session Title: Raman Spectroscopy Advances

Abstract Title: Miniature Deep UV Raman and Fluorescence Instruments for Trace Chemical and Biological Detection

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Abstract Text:
This paper describes new developments in miniature deep UV Raman and fluorescence instruments for detection of trace chemical, biological, and explosives (CBE) material on surfaces. Instrument examples include the newly selected Mars rover arm mounted SHERLOC (Scanning Habitable Environments with Raman and Luminescence for Organics and Chemicals) instrument to be flown to Mars in 2020, and the DOD Biological Reconnaissance & Analysis using Non-Contact Emission-spectroscopy (BRANE) sensor under development with the U.S. Army.

Detecting Raman signatures of CBE materials on natural surfaces is difficult. Making this detection in complex natural environments where fluorescence from analytes or surroundings in the Raman spectral region, or ambient light backgrounds are potential interferents or obscurants, is additionally difficult. One of the solutions is to perform excitation sufficiently far in the ultraviolet to avoid fluorescence within the Raman region or from ambient lighting and limit penetration depth to spatially separate analytes from substrates. This introduces a different set of problems due to the limitations of deep UV lasers and increases in sample damage.

To enable these measurements to be practical in a hand-held or robot-mounted sensor, significant improvement of the excitation and detection efficiency and miniature deep UV lasers has been necessary. We have improved the excitation and detection efficiency by over 1000x compared to traditional Raman systems. We will show results in which good signal-to-noise Raman spectra of a wide range of materials are achieved using only a few mJ of excitation energy. In addition, we will describe progress in the development of new, miniature (400 gram) lasers with output over 1 W at 248.6 nm and linewidth less than 0.5 pm. These lasers have been tested over a temperature range from -1350C to +700C and three times the shock and vibrations specifications for a Mars mission, without failure.

Keywords: Biosensors, Chemical, Fluorescence, Vibrational Spectroscopy
Application Code: Environmental
Methodology Code: Vibrational Spectroscopy
We report on the development of novel state-of-the-art deep UV Raman instrumentation and methodology for stand-off detection of energetic molecules. UV Raman spectra excited below 230 nm are free from fluorescence background. Deep UV Raman spectra of high explosive materials are resonantly enhanced. This enables explosives trace detection at a distance. We used recently developed a high power deep UV laser that produce ~100 mW of 213 nm light. This is solid state Nd:YVO4 pump laser designed and manufactured by UVIsIR Inc. This laser utilize novel technology to improve 5th harmonic BBO crystal durability. The laser head is compact in size and weights ~ 20 lbs. We designed a new high throughput, high dispersion UV Raman spectrometer. This spectrometer consists of high-efficiency filtering stage monochromator and high-dispersion Echelle spectrograph. High spectrometer throughput enables ultrahigh signal-to-noise ratio deep UV Raman spectra. We used this novel spectrometer to measure UV Resonance Raman spectra of PETN, TNT, NaClO4 and NH4NO3 at a stand-off distances > 2 m. We discuss the explosives stand-off detection limits and factors affecting them.
Raman hyperspectral imaging reveals both the chemical identity and spatial distribution of chemical domains within a sample. Conventionally, Raman imaging has been performed using point mapping, line-scanning or wide-filed imaging modalities. Raman is an inherently inefficient scattering method, however, and Raman imaging techniques often require long detector integration times and high laser powers to obtain suitably useful signal-to-noise ratios. Increasing the spatial resolution across a fixed region of interest can often lead to impractically long acquisition times. In the work presented here, we have developed a multi-megapixel digital micromirror device (DMD) based Raman imaging system that takes advantage of spatial multiplexing to provide improved signal to noise ratios at comparably shorter acquisition times. The method utilizes simplex encoding matrices to generate binary DMD masks and Gaussian elimination to reconstruct Raman images of the sample at each wavelength band. The encoded light is detected using a conventional imaging spectrograph, enabling all wavelength bands within the spectral range to be recorded in a single acquisition for each spatial mask. Because noise and other artifacts that lead to intensity-based errors can be distributed across all image pixels during data recovery, validation of the Raman DMD imaging system is important and has been performed on model samples using the point-mapping modality. In addition to the instrument design and performance metrics, several Raman image datasets from both model and complex real-world samples are presented.

Keywords: Bioanalytical, Raman, Vibrational Spectroscopy
Application Code: Other
Methodology Code: Vibrational Spectroscopy
Fast, quantitative analysis of bulk mixtures has become increasingly important in regulating pharmaceutical manufacturing processes. Raman spectroscopy is an attractive method for confirming concentrations of these materials as it can offer quick, non-destructive and highly selective quantification without the need for time consuming sample preparation.

Transmission Raman spectroscopy (TRS) presents significant benefits over the more common backscattering Raman Spectroscopy by providing an average through the entire sample, offering fast and effective analysis of content uniformity. In this work we introduce a novel technique utilising TRS and Renishaw’s high speed encoded stage to enable the analysis of bulk mixtures far larger than conventional single point TRS configurations (analysing tens of cm$^3$).

Analysis can be configured to provide fast average information or a chemical image revealing mixture distribution (spatial transmission mapping) over this massive area in just a few minutes.

The analysis of very large mixtures is critical to ensure uniformity and relative amounts are accurately quantified and optimised. Achieving this provides less variation when, for example, formulations are subsequently manufactured. This potentially could reduce issues with regulatory dosage testing of batches, where amounts and distribution are not accurately known until final analysis. At this point significant material write-off or damaging regulatory non-compliance could be avoided.

Both single and mapped TRS measurements of powder mixes with differing concentrations will be reported to demonstrate the benefit of analysing such large volumes on short timescales.

Abstract Text

Keywords: Raman
Application Code: Pharmaceutical
Methodology Code: Vibrational Spectroscopy
An assay has been developed using highly specific fluorescently labelled monoclonal antibodies which are conjugated to silver nanoparticles for the direct detection of small molecules using surface enhanced Raman scattering (SERS). SERS produces a unique ‘fingerprint’ spectrum in which the peaks are narrow, this has advantages for multiplexing, allowing for the detection of multiple target analytes. An antibody which has a high affinity for a target analyte is an attractive capture probe as it will specifically bind. Therefore, silver nanoparticles were functionalised with antibodies which, upon binding of the target analyte, allow for a specific SERS spectrum to be obtained due to the analyte being brought close to the nanoparticles’ surface. Proof of concept was demonstrated by using TNT and RDX.

TNT and RDX are small nitroaromatic molecules which are commonly used as concealed explosives. Therefore, a simple and rapid test for explosives which has an unambiguous result, is affordable and can be widely deployed is highly desirable. We have developed a direct SERS detection assay which is quantitative, fast, simple, highly specific and selective towards targeting molecules. This assay has resulted in an observed limit of detection of 10 nM of TNT and 600 pM of RDX. Furthermore, with the use of multivariate analysis, we can also detect and distinguish both targets from within a complex matrix. This approach demonstrates the use of sensors for fast, sensitive and direct detection of small target analytes.

Keywords: Biosensors, Detection, Immunoassay, Surface Enhanced Raman
Application Code: Nanotechnology
Methodology Code: Vibrational Spectroscopy
## Abstract Text

We developed novel wide-field Raman hyperspectral imaging spectrometers that utilize crystalline colloidal array photonic crystals to select narrow wavelength spectral regions for detection and imaging. Our photonic crystals are comprised of highly charged, monodisperse silica or polystyrene nanospheres that self-assemble in solution to form face centered cubic crystalline arrays. The photonic crystals diffract a narrow wavelength spectral interval governed by Bragg’s Law whose wavelength can be tuned by changing the incident angle of collimated light. We reduced the bandwidth of the diffracted spectral region by using recent advances in silica nanosphere synthesis to create smaller nanospheres with increased monodispersity. Directing a collimated beam of Raman scattered light onto our photonic crystal allows us to select a narrow wavelength spectral region for detection. Focusing this light onto a CCD chip produces a Raman image which spectrally details the chemical composition of the sample. We utilized our wide-field Raman imaging spectrometers to produce visible and deep UV Raman images.

### Keywords:
- Imaging
- Raman
- Spectrometer
- Spectroscopy

### Application Code:
- Other

### Methodology Code:
- Vibrational Spectroscopy
Handheld Raman is a powerful technique for material identification. It has applications in the pharmaceutical, cosmetics, and dietary supplements industries and others and is used by safety and security professionals. The primary advantage of using handheld Raman is to take the analysis to the sample rather than bringing samples to an analytical laboratory, improving turnaround time and efficiency. Measurements performed at a decision point outside a formal testing laboratory can reduce analysis time and cost. However, doing analysis at the sample is only viable if the handheld Raman analyzer can make reliable measurements.

We have investigated and implemented a variety of different ways to improve material identification using an advanced handheld Raman instrument. Improvements to material identification can be made by the careful selection of excitation wavelength, using advanced spectral search algorithms and improvements in spectral quality such as higher resolution, improved signal-to-noise and a broader spectral range. Examples showing improved material identification from different application areas will be discussed.

Keywords: Infrared and Raman, Instrumentation, Pharmaceutical, Vibrational Spectroscopy
Application Code: Pharmaceutical
Methodology Code: Vibrational Spectroscopy
A new Ultra-fast Raman Chemical Analyzer, AcuScan2000 has been developed for molecules identification and quantification of organic as well as inorganic substances. The AcuScan2000 is designed with the integration of robotic control of the whole processes in sample preparation, sample injection, laser positioning and detection, Raman maximum signal intensity optimization, spectrum collection, and spectra data comparison to more than 16,000 Raman spectra library data for material identification. With a proprietary spectra deconvolution technique in conjunction with the highly reproducible spectra intensity data and calibration relationship between spectra intensity and concentration of the measured substance, the system presents highly reproducible quantitative results to between 0.1 to 0.5% RSD for polystyrene, lipstick, EDTA, 10% aqueous sucrose solution, etc.

The Raman chemical analyzer can also become an on-line detection system for HPLC to obtain molecules identification and quantification for sample components eluted from HPLC column.

Applications of the AcuScan2000 for plasticiser, enviromental contaminants, Food contaminants, etc. will be reported.

Keywords: High Throughput Chemical Analysis, HPLC Detection, Raman, Vibrational Spectroscopy
Application Code: High-Throughput Chemical Analysis
Methodology Code: Vibrational Spectroscopy
Two biginelli compounds (1 and 2) were synthesized and further used for developing fluorescent organic nanoparticles (FON’s) using reprecipitation method. Compound 1 is derived from urea while compound 2 is derived from thiourea. Characterization techniques like NMR and MASS were used to characterize the compounds. The FONs were characterized using techniques like TEM and DLS. The FONs were checked for their sensing properties using fluorescent spectroscopy technique. Nano-aggregates of compound 1 did not show any sensor activity. On the other hand, nano-aggregates of compound 2 showed selectivity for mercury by showing enhancement in the monomer peaks of pyrene moiety after the addition of mercury. Moreover, the complex of nano-aggregates of 2 and mercury recognised the iodide ion by showing quenching in monomer and excimer emission. Mercury can be detected at a lowest concentration of 21 nM by nano-aggregates of 2. Also, detection limit of 2.Hg(II) for iodide is 4 μM.

Keywords: Biological Samples, Fluorescence, Nanotechnology, Sensors
Application Code: Biomedical
Methodology Code: Sensors
Polystyrene-core silica-shell nanoparticles (nPs) were developed for scintillation proximity assay (SPA) applications with \( \beta \)-particle emitting radionuclides in physiological samples and biological systems, where due to the high surface area and small volume, polystyrene-core silica-shell nP assays could have greater utility than other SPA designs. \( \beta \)-particle emitting radionuclides are frequently used as signal transducers in biological assays because unlike fluorophores or fluorescent protein tags, radionuclides do not significantly increase the size or mass of the labeled component, and therefore have minimal effects on binding, conformational changes, diffusion, and active transport. In addition, lower background signals are typically obtained for radioassays compared to fluorescence assays. For convenient quantitation, aqueous samples are usually dispersed in scintillation cocktails, which are mixtures of energy-absorbing aromatic hydrocarbons, surfactants, and scintillant fluors, and are consequently incompatible with most biological samples. SPA is a derivative of solid scintillation counting, wherein specific binding of radiolabeled analyte to a solid scintillator surface increases the probability of energy absorption by the scintillant. SPA is particularly useful for \( \beta \)-emitters with low penetration depths and results in an increased number of emitted photons upon analyte binding. SPA also eliminates the need to separate bound from unbound radiolabeled analytes, markedly enhancing the throughput and simplicity of assays. Furthermore, the entrapped scintillant fluors can be selected for photon emission at characteristic wavelengths. The addition of 15-25 nm thick silica shells to 150-200 nm diameter fluor-doped polystyrene core particles makes the particles hydrophilic and facilitates the covalent attachment of binding ligands to the exterior nP surfaces.

**Keywords:** Bioanalytical, Biosensors, Luminescence

**Application Code:** Bioanalytical

**Methodology Code:** Sensors
Plasmonic coupling, occurring between two metallic nanoparticles in close proximity, can produce “hot spots” and further enhance the local electromagnetic field. This phenomenon provides a great opportunity for fluorescence enhancement if a fluorophore is located within the intensified electric field. Few literatures have reported this effect between an anisotropic metallic array and a fluorophore. Here, to achieve desired plasmonic coupling, we demonstrated controlled gold nanorod (AuNR) assembly with well-defined surface plasmon pattern on a hydrophilic-hydrophobic substrate surface. Then, we investigated the distance-dependent fluorescence enhancement by introducing fluorescence-labeled DNA of different lengths to the assembled AuNRs. The maximum enhancement was observed at a specific distance between 0 and 30 nm for a given AuNR size. Besides the distance, the fluorescence intensity of a dye absorbed to AuNRs was strongly dependent on the overlap between LSPR of AuNR and the spectral properties of the dye. It is noted that the maximum fluorescence from a dye occurred when the LSPR of AuNRs was overlapped with both excitation and emission spectrum of the fluorophore. This plasmonic enhanced fluorescence is very promising in multiplex DNA detection. As fluorescence labeled DNA sequences hybridize with their complimentary sequences conjugated to AuNR assembly on substrate, the enhanced fluorescence renders a more sensitive detection than traditional micro-array.

Keywords: Biosensors, Fluorescence, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Sensors
Current techniques, such as MRI and microelectrodes, have elucidated much about brain morphology and function. However, it is still difficult to quantify the spatio-temporal fluctuations of neurotransmitter release in the synaptic cleft. One limitation is working in the tight architecture of the brain. We report on a novel DNA-dendrimer nanosensor to detect acetylcholine transmission in neural tissue slices. The advantage of using DNA enables us to design tailored dendrimer architectures, which efficiently assemble and support maximal co-localization of recognition and reporter elements. To detect acetylcholine, the design utilizes butyrylcholinesterase as a recognition element and a pH sensitive fluorescent reporter held with close proximity of one another. The DNA-architectures highly control and organize the spacing of these two moieties, which leads to increased sensitivity and brighter signal with a lower limit of detection of 200µM. The diameter of the nanosensors was observed to be 130±24 nm by transmission electron microscopy and the structure of the DNA architectures was confirmed by atomic force microscopy. The nanosensors responded dynamically and reversibility to acetylcholine in cells and brain slices. We show the nanosensor components and architecture report acetylcholine concentrations with high spatio-temporal fidelity and are sensitive and selective to acetylcholine in the neurophysiological range. We anticipate this work will further analytical capabilities of physiological processes in physically constrained environments.

This work is supported by DARPA and the NSF.
Surface enhanced Raman spectroscopy (SERS) offers considerable advantages as a transduction method for many types of optical sensors, due to its sensitivity and potential identification of multiple target species simultaneously. Over the years, SERS substrates have been developed to be capable of extreme sensitivities, with even single molecule detection having been achieved at randomly located hot-spots of colloidal aggregates. However, such architectures suffer from significant irreproducibility, due to the randomness of the aggregation. Alternatively, strategies such as ordered 2D arrays or enhancements based on single probes (e.g. immunno-nanosensors, nanostars) have high reproducibilities, but are generally limited in their signal enhancements.

To bridge this gap, we have developed a widely applicable enhancing geometry based on metal thin films interleaved with dielectric spacers that takes advantage of a third dimension (perpendicular to the surface) to enhance the signal independently from the underlying structure. Preliminary evidence into the mechanism of this enhancement has shown that the dielectric spacer material and thickness play a key role in the magnitude of the resulting additional enhancement. In this paper we investigate the material and thickness dependence of multilayer enhancement using substrates obtained with ultrathin oxide spacers generated by atomic layer deposition. The SERS sensitivity obtained with substrates fabricated from semiconductor materials as well as dielectric materials have been characterized in order to better understand which properties influence the multilayer enhancement the greatest. In addition a model to describe the mechanism by which the spacer properties influence the multilayer enhancement will also be discussed.
Abstract Text

We developed novel air-stable 2D polymerized photonic crystal (2DPC) sensing materials for visual detection of the vapor phase analytes water and ammonia by incorporating a new ionic liquid, ethylguanidine perchlorate (EGP) as the dispersing medium. 2D arrays of polystyrene nanospheres were embedded in poly(hydroxyethyl methacrylate) (pHEMA)-based polymer networks dispersed in EGP. The bright 2D photonic crystal diffraction depends sensitively on the 2D array particle spacing. The particle spacing change reports on the volume phase transition response of the polymer to the analytes. Water absorbed by EGP increases the polymer-solvent interaction parameter, so that at equilibrium the polymer is smaller. We discuss a novel transient volume phase transition that is observed at smaller time scales, where the polymer network swells in the first 20 minutes before relaxing to the equilibrium state. The process is reversible and the transient volume phase transition is observed for both increasing and decreasing water concentrations. Ammonia absorbed by EGP deprotonates the carboxyl group of the pHEMA-Acrylic Acid polymer, swelling the polymer and causing a redshift in the diffracted light. This ammonia sensor acts as a dosimeter since the volume phase transition is not reversible. We discuss the particle spacing rate of change that is associated with exposure to different concentrations of ammonia gas. This work was funded by the Defense Threat Reduction Agency (DTRA) grant no. 1-10-1-0044.

Keywords:
Gas, Materials Science, Polymers & Plastics, Sensors

Application Code:
Polymers and Plastics

Methodology Code:
Sensors
Development of analytical methods for highly sensitive, selective and fast detection of cancer cells and biomarkers is of great significance to early diagnosis and therapeutic assessment of cancers. In this paper, a highly selective and sensitive electrogenerated chemiluminescence (ECL) biosensor for the detection of prostate PC-3 cancer cells was developed using a prostate specific antibody as a capture probe and ruthenium complex-labelled wheat germ agglutinin as a signal probe. The ECL biosensor was fabricated by covalently immobilising the capture probe on a graphene oxide-coated glassy carbon electrode. Target PC-3 cells were selectively captured on the surface of the biosensor, and then the signal probe was bound with the captured PC-3 cells to form a sandwich. The ruthenium complex (Ru1)-labeled wheat germ agglutinin (WGA) employed as a signal probe was synthesised and characterised by UV-Vis absorption spectrometry and ECL method. Confocal laser scanning microscopy suggests that Ru1-WGA is attached to the receptors of WGA on the cell surfaces. The conditions of the biosensor fabrication and the capture probe concentration and the incubation times were optimized. The results showed that the ECL intensity of the sandwich biosensor was logarithmically directly proportion to the concentration of PC-3 cells over a range from 700 to 30000 cells/mL with a detection limit of 260 cells/mL. The ECL biosensor was also applied to detect prostate specific antigen with a detection limit of 0.1 ng/mL. The high selectivity of the biosensor was demonstrated in comparison with that using the ECL fabricated by wheat germ agglutinin as a capture probe and ruthenium complex-labelled wheat germ agglutinin as a signal probe. The strategy developed may be a promising approach and could be extended to the design of ECL biosensors for highly sensitive and selective detection of other cancer-related cells or cancer biomarkers using different probes.
**Detection of Clenbuterol by Using Localized Surface Plasmon Resonance**

We have been investigating the Surface Plasmon Resonance (SPR) sensing system. The angle-fixed typed SPR showed remarkably high sensitivity as the mass transducer. The achieved LOD was 30 pg cm^{-2}. However, the sensitivity for clenbuterol by using direct immunoassay was only 500 ppb (ng mL^{-1}), although the requirement was 100 ppt (pg mL^{-1}). Thus, the indirect competitive inhibition immunoassay was used for detection of clenbuterol. By this immunoassay, the sensitivity became 8 ppt (pg mL^{-1}). Kinetics study could explain the reason of this improvement. Moreover, kinetics also predicted the further improvement by using secondary antibody. Aiming to further signal amplification by not only the mass change effect but also localized surface Plasmon resonance (LSPR), Au nanoparticle was attached to the secondary antibody. As a result, the sensitivity at the secondary immunoassay became a thousand times greater than that of the first immunoassay. It was lower than ppq order (fg mL^{-1}).

The sensor surface structure was characterized by electrochemical mesurement (Cyclic voltammetry), FT-IR RAS, X-ray photoelectron spectroscopy, and Scanning tunneling microscope. We will discuss that the orientation of antigen (clenbuterol) highly affected on the sensitivity in the immunoassay format of indirect competitive inhibition based on kinetics study.

**Keywords:** Biosensors, Detection, Portable Instruments, Sensors

**Application Code:** Food Safety

**Methodology Code:** Sensors
Short chain fatty acids can be difficult to analyze by GC due to their highly polar nature affecting activity within the GC system, as well as affinity for polar solvents when performing headspace analysis. Additionally, if FID detection is used, acids like formic acid give exhibit low sensitivity due to the low number of C-H bonds. Esterification is often a technique used to derivatize fatty acids to esters using an alcohol to reduce polarity, eliminate activity, and increase sensitivity. Headspace analysis with in-vial derivatization provides an easy way to analyze fatty acids with minimal sample preparation. Within this technique there are several options in selecting the appropriate alcohol and ester to optimize analysis of the compounds of interest for different applications. Several alcohol / fatty acid combinations will be analyzed with this method to show suitability for headspace analysis.

Keywords: Capillary GC, Derivatization, Headspace, Volatile Organic Compounds

Application Code: General Interest

Methodology Code: Gas Chromatography
Stability, Linearity and Repeatability of Nitrogen and Carbon Determination by Flash Combustion Using Argon as Carrier Gas

Elemental analyzer with a thermal conductivity detector for Nitrogen and Carbon determination uses as typical Helium carrier gas due to the optimum sensitivity. However, in the last years, the possible worldwide shortage and high cost increasing of Helium, led to try an alternative gas. Argon gas, readily available with a reduced cost, is the alternative.

The FLASH 2000 Analyzer, based on the dynamic flash combustion of the sample, copes effortlessly with the wide array of laboratory requirements such as accuracy, day to day reproducibility and stability. The instrument was tested with Argon as carrier gas in comparison to Helium using the same Eager Xperience OEA dedicated software for the quantification of the Nitrogen and Carbon content.

This paper presents data on Nitrogen and Carbon determination of pure organic compounds in a large range of concentration in order to demonstrate the performance of the instrument using Argon gas in terms of stability, linearity, accuracy and repeatability.

Keywords: Elemental Analysis, Instrumentation, Method Development, Quantitative
Application Code: General Interest
Methodology Code: Other
GCxGC is one of the fastest growing areas in analytical chemistry due to its ability to resolve a large number of compounds, even in the most complex samples. It employs two columns in series, separated by a modulator. One key to the successful operation of GCxGC is that the two columns must have orthogonal selectivity, that is, they must utilize different retention mechanisms. Column selection strategies to achieve orthogonal selectivity include:

1. Non-polar to polar strategy. Analytes are separated on a non-polar column in the first dimension, and on a polar, highly polar, or extremely polar column in the second dimension.
2. Polar to non-polar strategy. Analytes are separated on a polar, highly polar, or extremely polar column in the first dimension, and on a non-polar column in the second dimension.
3. High resolution polar to non-polar strategy. Analytes are separated on a long (100 m) highly polar or extremely polar column in the first dimension, and on a non-polar column in the second dimension.

Details, benefits, and GCxGC plots for each of these three column selection strategies are presented.

Keywords: Capillary GC, Gas Chromatography, Gas Chromatography/Mass Spectrometry
Application Code: General Interest
Methodology Code: Gas Chromatography
A scanning, microfluidic platform was developed for use with a flow-based biosensor, affinity-based separations and monolithic columns containing composite nanohybrid materials. The system could be used with HPLC or syringe pumps for the application of mobile phases, reagents and samples, and included a programmable x-y-z platform for detection in three dimensions on devices that included microcolumns, disks, and microfluidic chips. A variety of detection formats were used with this platform, including a scanning near-infrared fluorescence microscope and fiber optic probes for fluorescence or absorbance detection. This system was used for detecting several model target analytes within the channels of microfluidic devices or on the surfaces of modified support materials. This system was also employed for affinity separations in which serum transport proteins were immobilized as binding agents within small monolithic columns, as well as use of similar proteins in flow-based biosensors for the detection of drugs in environmental or clinical applications.
New micro plasma emission detector for gas chromatograph using helium, argon or nitrogen carrier gas. System with unique design that improves sensitivity using argon or nitrogen carrier gas compared to any other plasma emission detector (PED). Selectivity obtained by using specific design to use up to 5 optical filters on one plasma cell to measure many impurities such as permanent gases, noble gases, toxic gases, light hydrocarbons, fluorides, corrosive gases, sulfurs, etc.
Gas chromatography has many positive attributes as an analytical tool, however its incompatibility with active compounds is certainly one of the biggest weaknesses and has not been fully overcome yet. The internal surface of a fused silica capillary tubing is chemically active and if not deactivated prior to making a GC column, it can cause solute adsorption and subsequent peak tailing. Therefore, the column making processes generally involve surface deactivation of capillary tubing prior to coating a stationary phase. Substantial progress has been made to make inert GC columns. Until recently, a Grob test mixture referred to here as a column performance test mixture (CPM) consisting of representative acidic, basic, aromatic, ester and hydrocarbon probe compounds was used to evaluate the column performance for inertness. In the current investigation, we have developed various deactivation processes to develop inert columns. Newly developed inert columns were tested using Grob CPM and also, more demanding strong probes “Über One” CPM. It is observed that an excellent performance of a column for Grob CPM does not necessary provide the best column inertness when compared to the column performance using strong probes Über One CPM. A comparison of the inertness of columns made using various deactivation techniques is illustrated using Grob and Über One CPMs.

Keywords: Capillary GC, Environmental Analysis, Gas Chromatography, Gas Chromatography/Mass Spectrometry
Application Code: Environmental
Methodology Code: Gas Chromatography
The Pulsed discharge ionization detector utilizes a pulsed DC discharge in helium as an ionization source. The PDD is a universal, non-destructive, high sensitivity detector particularly suited for the analysis of permanent gases. These compounds can be detected up to the low ppb with an excellent linearity range. A criticality in the analysis of gases is the introduction of the sample into the gas chromatographic system, the most reliable and wide-spread technique to inject a gas or a vapor is the injection through a sampling valve.

In this poster the benefits of the adoption of a modular gas chromatograph that offers a flexible and easily installable solution for gases analysis will be presented. Both the PDD detector and the gas sampling valve are comprised into two, user-installable, fully integrated modules that include the detector body or the sampling valve body along with the pneumatic circuits and the electronics necessary for their operation and control.

This modular design allows a new level of instrument flexibility, where inlets and/or detectors selection is based on the application in use, and can be changed in a matter of few minutes by the operator when a new analytical need or application requires different injector and/or detectors without affecting the analytical performances. This poster will discuss the excellent performance of such a system showing the data obtained on gas samples analysis.

Moreover, without adding further hardware complexity, the gas sampling valve module chosen can be easily set to backflush to vent the undesired part of samples, therefore offering an easy and integrated set-up for more complex analysis, without affecting the PDD response. Data showing performance of this solution will be illustrated and discussed.

Keywords: Air, Detector, Gas Chromatography, Instrumentation
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography
Characterization of chromatographic separations is dependent on many factors, including the detector chosen to monitor the separation. A single detector may not be suitable for analysis; it is often useful to combine orthogonal detection. It can be challenging to use detectors based on different physical properties in the same separation: the different detectors seldom have the same sensitivity or linear dynamic range. Also, it can be difficult to find a mobile phase that is suited to work with the mechanism of detection for both detectors. The mass spectrometry and photodiode array detectors give very different information about a sample component. The concentration required for the PDA analysis is usually at a higher intensity than the MS linear response. We have developed a split-and-dilute system to address these challenges. The use of this device is illustrated with a combination of MS and PDA detection. A passive splitter is used to sample a fraction of the flow from the column effluent. The bulk of the flow continues to the optical detector while a portion is then mixed with a makeup liquid that carries the diluted sample to the MS. Using a passive split ratio ensures that both detectors provide and maintain a linear response. The makeup flow can be used to provide more MS compliant conditions for optimum detection. For example, MS signal intensity can be suppressed by ion pairing additives in the mobile phase. We will show elimination of the suppression caused by such modifiers while retaining the desired chromatographic behavior.

Keywords: Analysis, Chromatography, Detection, Liquid Chromatography/Mass Spectroscopy
Application Code: General Interest
Methodology Code: Liquid Chromatography/Mass Spectrometry
Although reversed-phase columns (e.g. C18) are most commonly used in a broad range of applications, they often fail to retain highly hydrophilic molecules (e.g. counterions), and offer limited selectivities. Mixed-mode chromatography provides a viable solution to these challenges by combining both reversed phase and ion-exchange retention mechanisms. One major advantage of this approach is that column selectivity can easily be modified for optimal selectivity by adjusting mobile phase ionic strength, pH and/or organic solvent concentration. As a result, not only is the selectivity of a mixed-mode column complementary to that of reversed-phase columns, but it also allows for the development of multiple complementary selectivities on the same column under different appropriate conditions. Mixed-mode chromatography is well-suited to retaining ionic analytes, whether hydrophobic (e.g. Naproxen) or hydrophilic (e.g. Na+ and Cl- ions), and requires no ion-pairing agents in the method, significantly improving the MS compatibility. Most importantly, mixed-mode chromatography column chemistry can be customized to a desired selectivity during stationary phase design. This presentation will give an overview on the latest mixed-mode chromatography technology, describe unique chromatographic properties of mixed-mode columns, and discuss analytical challenges that have been addressed by mixed-mode chromatography approach. Examples include determinate on of pharmaceutical counterions, simultaneous separation of anionic, cationic, nonoionic and amphoteric surfactants, high resolution and fast LC-MS analysis of paraquat and diquat, and analysis of glycans from proteins and human IgG.
A gaseous amine introduction device, placed after a conventional suppressed conductivity detector in an anion chromatography system based on hydroxide eluent, converts the suppressed eluents from the acid form to an ammonium salt (NR₃ + HX → NR₃H⁺ + X⁻). Diethylamine is chosen as the amine source due to its low pKb value (pKb 2.91) and high vapor pressure. A conventional suppressed anion chromatography system produces a poor response for very weak acids (pKa >7), if at all, because HX is hardly ionized. A second conductivity detector then measures the conductivity again. The eluents are thus detected against a low diethylamine background (typically 20-30 [micro]S/cm) as negative peaks, because the equivalent conductance of OH⁻ is greater than X⁻. The performances of the amine introduction device were evaluated with respect to induced dispersion and background noise. The introduction of diethylamine in the vapor phase directly through a permeable membrane actually is superior to solution phase NaOH introduction. Relative to a microelectrodialytic NaOH introduction previously reported, the amine introduction device is simpler to build and use. The availability of such a device is expected to facilitate the use of 2-D detection.

Keywords: Detection, Ion Chromatography, Ion Exchange, Method Development
Application Code: General Interest
Methodology Code: Liquid Chromatography
Injection is one of the most critical functions for the performance of gas analysis systems based on gas chromatography. The aim of this study was to assess the performance of different gas injection valves for portable and fast GC application. Mechanical valves from two different constructors, with internal and external volume were tested and compared to a silicon injector valve.

Experimental procedures:
The work presented here compares the efficiency of each valve determined with GCAP, a fast and portable GC analyzer based on silicon technology, developed by APIX Analytics. GCAP awarded the coveted Bronze Award for ‘Best New Product’ at Pittcon 2013 in Philadelphia. It is equipped with a silicon injector and two sensors: a µTCD and a NEMS (Nano Electro Mechanical System) connected in series. The peak’s shape and width will be compared, as well as the plug & play feasibility, the size and the lifetime of each injector type.

Results and conclusions:
Results will demonstrate the importance of injection on the performance of gas analysis systems. High efficiency of silicon injector, resulting in faster throughput, smaller sampling size, higher sensitivity, and easier maintenance, totally confirms the advantage of using silicon technology for fast and portable GC systems. This study conducted in collaboration with Air Liquide, show in particular the advantage of this technology for the calculation of BTU in natural gas application.

Acknowledgement to the source of funding that supported the work: APIX Analytics, Air Liquide

Keywords: Fuels\Energy\Petrochemical, Gas Chromatography, Nanotechnology, Portable Instruments
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography
A novel on-column gas detector capable of measuring the transient partition coefficient is developed based on a dual-Fabry-Pérot-cavity structure for micro gas chromatography. The detector is fabricated by inserting an optical fiber coated with a layer of gas sensitive polymer on its endface into a hole drilled on the top of a microfluidic channel which is formed by anodic bonding a glass slide incurved with a line of square groove onto a silicon wafer. The hole is sealed afterwards to prevent any sample loss. The light coupled into the optical fiber is partially reflected at three interfaces of fiber endface-polymer, polymer-air and air-silicon wafer, respectively. The first and second interfaces, and second and third interfaces generate two sets of interference spectra, corresponding to a polymer and an air Fabry-Pérot (FP) cavity. When it is injected into the channel and travels through the detection point, the analyte will be distributed into both of the two FP cavities, resulting in the change of their optical paths and thus the shift of their interference spectra. Therefore, by monitoring their interference spectra shift, the transient amount of the analyte distributed in both FP cavities can be measured, which can be directly used to calculate the transient partition coefficient of the analyte.

Keywords: Gas Chromatography, GC Detectors, Lab-on-a-Chip/Microfluidics, On-line
Application Code: Clinical/Toxicology
Methodology Code: Gas Chromatography
A number of natural materials that possess significant properties (e.g., low cost, easily manipulation for ligand attachment, etc.) have been used for biochromatography1. Among them, pumice has extra properties such as more stability and large surface area2. In this study, chromatographic performance of Cu2+-attached pumice particles embedded to monolithic cryogels (Cu2+-APPsEMC) for human serum albumin (HSA) was investigated. Monolithic composite cryogels (MCCs) were prepared by means of the polymerization of gel-forming precursors at subzero temperatures. Poly(2-hydroxyethyl methacrylate) (PHEMA) and N,N´-methylene-bis-acrylamide (MBAAm) were used as monomer and cross-linker, respectively. The chemical composition of pumice was determined by XRF. Surface morphology of the composite cryogels was investigated by SEM. Embedded particles (15 mg) in cryogel column were used in the adsorption/desorption of HSA from aqueous solutions. The effects of pH, ionic strength, initial HSA concentration, temperature and flow rate on adsorption were investigated. The maximum HSA adsorption was observed as 549.5 mg/g pumice at pH 8.0 phosphate buffer with initial HSA solution of 3 mg/ml. The efficiency of HSA adsorption from human serum, before and after albumin adsorption was also investigated with SDS-PAGE analyses.

Chitin is the second most abundant biopolymer in nature after cellulose. Problems related to dissolving chitosan in neutral or basic media restrict its usage in many fields. To enhance the solubility of chitosan in aqueous solutions, water-soluble chitosan derivatives are synthesized via the reactive hydroxyl and amino groups that are already present on the chitosan polymer.

In this study, for the purpose of eliminating the solubility problem of chitosan, and thereby extending its usability firstly, a water-soluble O-carboxymethyl chitosan and its cupper complexes were synthesized. Then, monolithic composite cryogels of poly(2-hydroxyethyl methacrylate) (PHEMA) and water-soluble chitosan complexes were prepared in a plastic syringe. And these Ni2+-attached composite cryogels were used for lysozyme adsorption from aqueous solutions. Optimum conditions of adsorption experiments in aqueous solution were performed at pH 8.0 as 129 mg/g composite. It was observed that lysozyme could be repeatedly adsorbed and desorbed with this composite cryogel without significant loss of adsorption capacity.

For the purification of large molecules, cryogels are an alternative stationary phase to particle-based media. But, due to the drawbacks of cryogels (i.e., low surface area) and particle sorbents (i.e., pressure drop in fixed beds), in recent years, the use of hybrid cryogels has greatly increased.

In this study, a novel hybrid cryogel column was synthesized for the purification of α-amylase from aqueous solutions. Firstly, poly(2-hydroxyethyl methacrylate) beads (2 mm size) were prepared, and after iminodiacetic acid (IDA) immobilization, Cu2+ ions were attached via the IDA chelating groups. These arranged Cu2+-attached PHEMA beads (Cu2+-ABs) were located into PHEMA based cryogel in order to prepare Cu2+-ABs embedded supermacroporous hybrid cryogel (Cu2+-ABsEHC) column. The specific surface area of the hybrid cryogel was found as 95 m²/g by using BET. The amount of IDA on beads was found as 875 mol IDA/g. It was approached to the optimum adsorption levels at initial α-amylase concentration of 5 mg/mL and pH 5.0 in Tris-Base buffer as 107.1 mg/g beads. It is demonstrated that this column is a potential separation medium for purification of α-amylase.


**Abstract Text**

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**Keywords:** Adsorption, Immobilization, Protein, Separation Sciences

**Application Code:** Bioanalytical

**Methodology Code:** Separation Sciences
A convenient, energy efficient continuous flow extractor is described. As the latent heat of vaporization is equal to the latent heat of condensation, these basic physical processes are combined in a unique heat exchanger. A compressor reduces the gas pressure within the evaporator allowing the solvent to vaporize and separate from solution and return to the condenser side compressed and be converted back into solvent. This enables the collection of the desired extract, solvent free and permits the continuous flow the regenerated solvent to continuously extract the desired sample. This extraction process requires no heat or cooling input and recycles all solvent. The only energy requirement is power for the compressor.
Advances in Separation Sciences

Quantitative Technology Alternative to GCxGC for Complex Mixtures

One-dimensional gas chromatographic (GC) analysis suffers from limited peak resolution for complex, compound-rich (100+) mixtures. Thus, addition of a second-dimension (2D) separation is often required for an additional separation mechanism. Conversion of 2D data to qualitative or semi-quantitative concentration results requires complex mathematical analysis which is difficult to maintain when chromatographic retention time shifts occur.

MAX™ is a new detection technology for GC which overcomes the qualitative and quantitative limitations of GCxGC. The novel optical detection system employed in the GC-MAX™ technology has proven calibration stability of better than 5% and < 1% RSD on repeat measurements. Once the MAX™ technology is calibrated for a compound, that calibration can be used for the life of the system or any similarly configured MAX™ technology system.

In addition to being fully quantitative, peak identification is also easily performed on co-eluting compounds by a novel spectral analysis algorithm based on chromatographic retention indices. The capabilities of this new technology will be demonstrated with ASTM D5501 Test Method for the Determination of Ethanol Content of Denatured Fuel Ethanol and ASTM D4815 Test Method for the Determination of MTBE, ETBE, TAME, DIPE, t-Amyl Alcohol and C1-C5 Alcohols in Gasoline. Here the advantages of the MAX™ technology to both quantify the high concentration ethanol concentration along with the low level oxygenates in a single analysis without the use of a second-dimension separation to remove hydrocarbon interferences/co-elutions will be discussed.

Keywords: Fuels\Energy\Petrochemical, Gas Chromatography, GC Detectors, Spectroscopy
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography
Matheson as a company is always striving for continuous improvement and development of new capabilities. Since customers demand the highest quality of materials readily available to them, there are many different ways to steadily improve. One method includes but is not limited to, the use of a measurement capability assessment (MCA) study. MCA studies encompass the measurement of attributes that are important to instrument capabilities. Data is obtained to demonstrate accuracy, static repeatability, linearity, as well as a Gage R&R study. Static repeatability is based primarily on measurement to measurement variation. The linearity study is used to determine the recovery range of the system in question which is done by using a set of standards which cover a concentration range of interest from high to low concentration. Static repeatability is a measure of the short term instrument variation. A gage R&R study measures total process variation and is performed by two operators who perform multiple trials on multiple standards over multiple days. These multiple trials estimate repeatability while the multiple operators are needed to estimate reproducibility. Multiple standards are needed to provide an estimate the variation. This MCA study involved the use of low percent level oxygen balanced helium as the material with the main subject of interest being a target concentration of 0.5%. Other standard concentrations included 0.2%, 0.32%, 0.75%, and 1% oxygen/helium mixes. The results from these studies will be presented and will be compared against the criteria for this type of mixture.

Keywords: Chromatography, Data Analysis, Detection, Gas Chromatography
Application Code: General Interest
Methodology Code: Gas Chromatography
Method Capability Assessment, or MCA, is a series of studies to determine if an instrument is suitable for a particular analysis and in this case is suitable for the measurement of a gas mixture at a given concentration, or a range of concentrations. A MCA study includes linearity testing, static repeatability, accuracy testing, and Gage R&R. A comparison study was also completed in this investigation to validate the concentration of the mixtures. The instrument used for the MCA was a Varian CP4900 Micro Gas Chromatograph equipped with a Thermal Conductivity Detector (TCD). The TCD detector was selected because of its simplicity in terms of response and wide diversity of concentration ranges that can be measured. A method was developed for the analysis of low percent level nitrogen mixtures in helium, and was validated using these MCA studies. The MCA study concluded the instrument was linear in the range of concentrations analyzed, repeatable and accurate, and the data could be reproduced over a range of concentrations, days, and operators.

Keywords: Data Analysis, Gas Chromatography, GC, Validation
Application Code: General Interest
Methodology Code: Gas Chromatography
Advances in Separation Sciences

Standard Materials Calibration System for the Expansion of the SI-Traceable Calibration Standard Mixture

Metrological traceability of mixed standard solution for calibration is required to sustain QA, QC and laboratory accreditation. This traceability to the international system of unit (SI) is described by combination of the unit of amount of substance (mole) and the unit of mass. Those SI base units are managed by National Metrology Institute (NMI). Mole is realized by NMI to assign specific value (concentration with uncertainty) to the pure substances. Number of pure substances assigned by NMI are limited, thus the supply of the SI-traceable Primary Reference Material (PRM) are not enough to satisfy the requirement of the quantitative analysis. We developed new standard materials calibration system with National Metrology Institute of Japan (NMII). The user of this system can expand SI-traceable standard mixture (gas or liquid) by calibration using gas chromatograph (GC) with PRM. By the conventional way, it is required to match the calibration components and the analyte. The point of this new system is that it does not need matching to the calibration substances and the analyte.

In particular, standard mixture (PRM) prepared by the gravimetric method from more than three pure substances (PRM) which assigned specific value by NMII is used for calibration on this system. This system equips the reactor between separation column and flame ionization detector (FID) on GC. After separation, each substance of this PRM is converted to CO[sub]2[/sub] and then reduced to CH[sub]4[/sub]. Each substance is detected as CH[sub]4[/sub] on FID so that calibration curve for atomic concentration of carbon as CH[sub]4[/sub] is obtained. The conversion efficiency of the reactor is checked if this calibration curve is drawn straight and goes through the origin. When unknown substances are measured in the same way and assigned specific value by this calibration curve, they can be assured SI-traceability.

We will show the way to expand SI-traceable standard mixture by this system.

Keywords: Calibration, Gas Chromatography, Quality Control, Reference Material
Application Code: Quality/QA/QC
Methodology Code: Gas Chromatography
Computational simulations of equi-potential contours and ion beam shapes in planar drift tubes were used to examine the influences of field strength on beam broadening in air at ambient pressure from 15 to 45 V/mm. Beams were shaped with a slit aperture placed in the drift tube cross section. Results from computational simulations with SIMION 8 demonstrate that beam broadening is decreased in y-axis with increased electric field in the drift region. While much of this control can be attributed to ion residence time in the drift region, shape of beams in non-linear fields suggests some focusing of ion beams with convex equi-potential contours. A planar drift tube was used to bend ion beams 90 degrees in a 14.3 mm radius in three beams passing center, outer and inner tracks of the bend. Differences in electric field with each track and additional time of travel could account for y-axis broadening of the beams. Images of the ion beams inside the planar drift tube were obtained with IonCCD array detector to support the simulations.
Response in an ion mobility spectrometer equipped with a beta emitter as the ion source originates in positive polarity through an ion-dipole or ion-induced dipole association between a neutral vapor analyte and a hydrated proton in a purified air atmosphere. This reaction forms product ions derived from the analyte and such ions appear as peaks in a mobility spectrum provide the product ions have lifetimes which exceed the time of residence in the drift tube. Existing understandings of quantitative response in IMS arise significantly from results of API MS studies. These suggest that quantitative response in an IMS drift tube at ambient pressure will be affected by moisture in the supporting gas atmosphere and that increases in moisture will cause decreases in absolute response across the range of dipole strengths. Most commercial hand-held or benchtop IMS analyzers contain sieve packs or membrane inlets to reduce and control moisture though effects have never been quantitatively established. Response factors obtained over a concentration range from limits of detection to source saturation are shown for molecules of carbon number from three to eleven for alcohols, aldehydes, esters, ketones, amines, and organophosphorous compounds. Effects are compared to computationally derived values of hydrate numbers for the reactant ion, a hydrated proton, at temperatures from 100 to 250°C. Implications for inter-comparison of quantitative response for IMS analyzers will be discussed.

Keywords: GC, Quantitative, Spectrometer, Water
Application Code: Other
Methodology Code: Separation Sciences
The capabilities of modern Wavelength Dispersive X-ray fluorescence (WDXRF) systems are rapidly advancing to meet the analytical demands in research areas from geological to biological. XRF is a non-destructive technique, and having the ability to characterize samples after elemental determination is essential for various research areas.

WDXRF has the ability to utilize multiple measurement techniques for elemental determination depending on sample type. The use of empirical calibrations using reference standards or standardless analysis where in most cases reference standards are not available for XRF, are options to meet qualitative or quantitative needs. Additionally, the WDXRF incorporates unique tools such as sectored inserts to accommodate low sample volume and 2D/3D imaging through Mapping/Spotting techniques.

Samples from research areas including geological, biological, process control, cosmetics and reclamation were analyzed for elemental composition and in some instances mapping was performed to determine elemental distribution across the sample. Each research area presented a unique sample type where extraction or digestion would be difficult to achieve, costly, and time consuming. The sample preparation for WDXRF analysis on these samples was minimal and cost effective.

Results demonstrate the power and flexibility of a modern WDXRF across diverse sample types. The 2D/3D imaging feature displays elemental distribution across a sample, which is beneficial for applications such as reclamation and biological analysis. For certain samples, comparative techniques (ICP-MS, ICP-OES, and XRD) were performed.

In conclusion, a calibrated analytical routine is the typical approach to many applications, standardless analysis methods can provide both valuable and accurate information where reference standards simply do not exist. The ability to examine and even quantify small and irregular samples or samples in-situ has also proven beneficial in many fields.

Keywords: Biological Samples, Cosmetic, Elemental Analysis, X-ray Fluorescence
We will describe a method for characterizing varnishes that may be applied to the more accurate restoration of artistic objects. In the first part of this study, a method based upon Raman Spectroscopy was used to develop a database of spectra for common varnishes, their degradation products, as well as a set of carboxylic acids and fatty acids that are typically found in aged varnish samples. A pattern recognition method based upon Singular Value Decomposition (SVD) was developed to determine how well the database represented the unknown varnish samples. SVD applied to the database yielded seven singular values that were then used to identify unknowns – Linseed Oil, Chinawood Oil, Dammar, and Mastic. The relative root-mean square (RMS) error for the unknowns was 1.7%, 1.7%, 4.9%, and 6.4%, respectively. In the second part of this study, the Raman method was applied to the products created in an accelerated aging chamber as a means to better understand the decomposition processes that occur in varnishes. An ageing chamber was designed and fabricated to allow for the precise control of temperature, light, and atmosphere. Time course studies of Linseed Oil and Chinawood Oil samples were performed over a range of thermal and photochemical conditions. Thermal studies were conducted from 25°C to 210°C over periods ranging from 2 to 170 hr. A direct correlation was found between the changes in the vibrational bands that were observed in the Raman spectra and the observation of overt physical changes in the samples. Based upon these data, several reaction mechanisms for the decomposition of the varnishes will be proposed.

Keywords: Art/Archaeology, Materials Characterization, Method Development, Raman
Application Code: Art/Archaeology
Methodology Code: Vibrational Spectroscopy
Abstract Text
Compared with other elemental analysis techniques, energy-dispersive X-ray fluorescence (EDXRF) is more suitable as a non-destructive method for precious museum objects. Recently, the research has been focused on portable HHXRF due to its convenience. In this study, Particle-induced X-ray Emission (PIXE), Bench top XRF and handheld XRF are applied on the analysis of ancient Chinese ceramics. The instrumentation capability and the analytical performance were systematically evaluated in this study.

Keywords: Art/Archaeology, X-ray Fluorescence
Application Code: Art/Archaeology
Methodology Code: X-ray Techniques
Portable X-ray fluorescence (pXRF) offers an opportunity bring the lab to the sample. As a non-destructive analysis tool that is also portable, it can be used to study the deteriorated 18th century frescoes of the Alamo in great detail. The objective is to digitally recreate the once-elaborate designs of these frescoes and reimagine a cornerstone of Texas history. Select areas of interest are analyzed with the pXRF, which identifies elements based on characteristic differences in energy emissions. We are particularly interested in identifying elements that are characteristic of pigments used in 18th century. Trace levels of copper, iron, lead, and mercury are particularly informative, indicating the possible use of verdigris, red and yellow ochres, lead whites, and vermilion pigments respectively. The presence of elevated counts of these elements, supported by multispectral imaging, and analysis of physical samples with scanning electron microscopy, provides a means of digital reconstruction of the original appearance of the Alamo. Thus far, several designs in the former mission’s sacristy have been well characterized and mapped, including a chalice-like shape now nearly invisible to the eye. In addition, multiple leaf and stem-like patterns that are only visible under ultra violet light have been confirmed with pXRF. This research has revealed repeated design elements and elaborate detail on the frescoes in the Alamo sacristy, moving us closer to a recreation of these nearly 300 year-old pieces of art history.
Raman spectroscopy is a valuable tool in the arsenal of the art conservationist. Through the acquisition of a vibrational spectrum of the surface of a sample, various types of important information can be gleaned. Of particular interest are chemical composition of colorants (inorganic vs. organic), potential origin of the minerals used in pigments, and what weathering or environmental damage has occurred, for example. Additionally, this information helps to establish authenticity and provenance of paint and pigment substances. This poster will focus on our efforts to develop more effective measurement methods for the challenging pigments that are encountered in older artifacts, especially those with green and blue-green mineral pigments, which have been found in the past to have a forbidding amount of native fluorescence.

The samples that will be of chief interest will be pigments on fresco samples from the Coriglia archaeological site near Castel Viscardo, Umbria (Italy). Data will be collected at 785 nm and background corrected to remove fluorescence commonly associated with Raman measurements of pigment materials. The resultant spectra will be compared with pigment and mineral databases, as well as mineral pigments purchased from art supply dealers.

**Keywords:** Art/Archaeology, Raman

**Application Code:** Art/Archaeology

**Methodology Code:** Vibrational Spectroscopy
A variety of demonstrated ethylene antagonists and agonists were modeled using Spartan’10 (Wavefunction, Inc) to develop a systematic theoretical basis for the identification of potential novel ethylene antagonists. It was found that known antagonists yielded HOMO energies at or above the calculated value for ethylene. It was also found that the known antagonists have LUMO energies lower than ethylene. Subsequently a variety of 1-substituted cyclopropenes were modeled and their orbital energies calculated. It was observed that as the electron donating ability at the 1-position increased, so did the HOMO energy. Strongly electron withdrawing 1-substituents had HOMO energies greater than ethylene and as such are predicted to exhibit ethylene antagonist behavior. This comparison of the empirical and theoretical data suggests a relatively simple methodology for predicting the antagonistic behavior of novel candidate compounds.
A theoretical investigation of trends in the HOMO to LUMO transition energies of tris(2,2’-bipyridyl)ruthenium(II) complexes containing various substituted ligands were performed using PM3 (semi-empirical) and ab-initio levels of calculation. Selected molecules included single and multiple substitutions at one, two and three bipyridine moieties. The effects on HOMO and LUMO orbital energies differences were studied as a function of substitution. Substitutions included varying the numbers and types of halogens on the bipyridines. In addition the effect of the addition of conjugated propenes to the bipyridine was investigated and compared to changes observed by the addition of saturated chains. A complex behavior attributed to both inductive and conjugation effects was observed for these modelled complexes. Substitution of hydrogen by fluorine was particularly anomalous. However, the substitutions did not affect the HOMO or LUMO orbitals’ essential ligand-based $\pi$ character.

Keywords: Computers, Fuels\Energy\Petrochemical, Spectroscopy
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Computers, Modeling and Simulation
Electron Transmission Through Thin Membranes for Non Radioactive Ion Sources

Beta radiation is an attractive ionization technique e.g. for Ion Mobility Spectrometry due to its reliability, well known ionization mechanisms and its independence from external power supply. A main drawback is that beta radiation sources are subject to different national and international regulations. Thus, the use of alternative ionization methods is desirable not only to add new detection options, but also to circumvent the strict regulations that apply for radioactive material.

The new non radioactive ion source consist of an electron gun operated under ultra-high vacuum (UHV). The generated electrons can be accelerated to a kinetic energy in a range from 1keV to 15keV. The resulting beam has enough energy to trespass a thin membrane, which separates the UHV region from the reaction region under atmospheric pressure were the ionization takes place. As an option the electron beam can be guided through the membrane into a chamber filled with a noble gas were so called excimer are generated and during the decay process Vacuum Ultra-Violet (VUV) photons of an energy characteristic for the composition of the excimer are emitted. In this way a dual ion source, electron impact and soft ionization is possible.

Monte Carlo calculations were conducted to simulated the electron transmission through the membrane. Electron distribution and the transmission efficiency in dependence of the initial kinetic energy of the electron beam was computed. The energy deposition in the thin membrane was evaluated.

The total energy deposition serves as input for the thermo-mechanical simulation of the thin membrane. In addition the load due to the pressure differences are considered.

As a result threshold values were calculated at which a mechanical destruction of the membrane will occur. The results are in good agreement with experimental observations. Also optimal operating parameters for the non radioactive ion source were determined.

Keywords: Membrane, Particle Beam
Application Code: General Interest
Methodology Code: Computers, Modeling and Simulation
The Brazilian chemistry industry trade balance is very alarming, as it presents increasing and persistent trade deficits since the 1990 decade. It reaches a new record in 2013, when it totaled US$ 32.0 billion with a 13.6% increase compared to 2012 (Abiquim, 2014).

Pharmaceutical products - which provide a more inelastic demand to Gross Domestic product (GDP) - most strongly impact this deficit. However, Brazil’s pharmaceutical industry is still currently dependent on imports and registered a deficit increasing of 15.3% between 2012 and 2013, jumping from US$ 6,637 billion to US$ 7,658 billion in 2013 (Abiquif, 2014).

The research was based on universe of 625 chemical products classified as pharmaceuticals (APIs) and medicines for human use, selected from the PRODLIST-Indústria 2010 (Ibge, 2011) for which it was collected the trade balance statistics (exports and imports) from AliceWeb System (http://aliceweb.desenvolvimento.gov.br) from 2009 to March, 2014.

The results points to a data base of 521 products - 228 drugs and 293 Active Pharmaceuticals Ingredients (APIs). Figure 1 shows the trade balance evolution in this period which shows negative results over the years as the small export growth is not enough to reduce the deficit caused by a growing on imports.

Table 1 details TOP 5 values (in US$ FOB) trade deficit in 2013, split by APIs and medicines for human use segments. The initial exploratory results points to future definitions of strategic medicines and APIs in order to revert the Brazilian dependency on imports in medium and long term. These findings should be investigated in the continuity of the present study, incorporating data from production capacity and planned investments in the sector.

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**Keywords:** Data Analysis

**Application Code:** General Interest

**Methodology Code:** Data Analysis and Manipulation
Electrochemistry

Selective Polymer Thin-Film for Spectroelectrochemistry of Rhenium Complexes

Film uptake, electrochemistry and spectroelectrochemistry of various rhenium complexes are investigated. The rhenium complex [Re(CO)3(H2O)3]+ is converted into the optically emissive complex [Re(CO)3(L-L)]+, where the ligand comprises of various bi-dentate ligands. Uptake is observed electrochemically using a selective polymer thin-film on an optically transparent electrode (OTE). Preconcentration of the complexes is observed optically by luminescence modulation. Optimization of the polymer film, electrochemical, and optical components allows for the modulation of complexes with various charges.

Keywords: Electrochemistry, Fluorescence, Luminescence, Spectroelectrochemistry

Application Code: General Interest
Methodology Code: Electrochemistry
Glassy carbon electrode was modified by drop-coating technique with aza-15-crown-5 for determination of Cu(II) by square wave anodic stripping voltammetric (SWASV). The new electrode exhibit an increase of sensitivity due the complexing capabilities of aza crown ether with metals cations. Best result were obtained with aza-15-crown-5 0.031 mmol L-1; pH 4.0; deposition potential -0.7 V and deposition time 80 s. A detection limit was 0.021 [micro] L-1, and linear range was 10-50 [micro]L-1. Interference from metal ions like Fe(II), Pb(II)y Cd(II) was also studied. The method is sensitive, selective and simple with a relative standard deviation of 11%.

Keywords: Chemically Modified Electrodes, Electrochemistry, Electrodes, Stripping Analysis
Application Code: Environmental
Methodology Code: Electrochemistry
Adenosine plays a major neuromodulatory role in the brain, impacting processes such as neurotransmission and blood flow. Spontaneous, transient adenosine release has been characterized in the rat brain using fast-scan cyclic voltammetry (FSCV) at carbon fiber microelectrodes. Unfortunately, several other analytes, such as histamine, ATP, and H2O2, have cyclic voltammograms that can appear similar to that of adenosine. Waveform manipulation has become an appealing route as it requires no additional materials and is simple to test in vitro. Recently, it was found that a sawhorse waveform could differentiate adenosine from ATP and H2O2 due to the presence of an extra characteristic ‘hump’ in the oxidative peak of adenosine. Histamine, unfortunately, had the same feature and could not be discriminated from adenosine. In this work, the traditional triangle waveform was slightly altered to include a 0.75ms hold at 1.25V during the anodic scan. Since histamine is oxidized at a slightly lower potential than adenosine, the hold allows histamine to completely oxidize before adenosine begins to be oxidized and thus, separates the peaks. Exogenously applied histamine and adenosine were separated with this method in a rat brain slice as well, though the hold potential had to be raised to 1.35V. Future work with this waveform includes applying Principal Components Analysis to determine concentrations of adenosine and histamine in mixtures. This will determine whether quantitative co-detection of these analytes can be performed. It may then be possible to explore the role of adenosine in neuromodulation of histamine.
Electrochemistry

Tuning the Structure of Ionic Liquids to Increase the Width of the Electrochemical Window

Supercapacitors store electrical charge in a double layer at the interface of an electrolyte and a surface, primarily porous carbon with high surface area. The energy density (ED) of such a capacitor is determined by $ED = \frac{1}{2} CV^2$, where $V$ is the potential difference between the plates of a capacitor and $C$ is the capacitance density. Therefore, extending the operational voltage of such devices, which is limited by the electrochemical window of the electrolyte or solvent, can improve the device energy density.

Typically, the electrochemical window of an electrolyte is limited by oxidation of the anion at positive voltages and reduction of the cation at negative voltages. For determining electrochemical stability, we used a recently developed method that involves linear regression in the approximately linear ranges of the linear sweep voltammogram. The new method is much less sensitive to variations in ion mobility and electrolyte concentration than the traditional method based on arbitrary cutoff values of the current density. We studied the effect of cation structure (aromatic vs. saturated) and straight or cyclic alkyl substituents attached to a nitrogen on the stability towards reduction. Even though aromatic cations, such as imidazolium ions, are largely used as ionic liquids in supercapacitors, our finding indicates that saturated cations (e.g., tetraalkylammonium cations) are more electrochemically stable. It was found that symmetry and steric hindrance of substitutes on the quaternary ammoniums does not improve electrochemical stability. Future work is focused on understanding how electrochemical stability can be correlated to molecular properties such as molecular orbital energy levels.


Keywords: Analysis, Electrochemistry, Fuels\Energy\Petrochemical, Voltammetry
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Electrochemistry
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<tr>
<td>Abstract Title</td>
<td>Gold Nanoparticles Modified Electrodes for Anodic Stripping Voltammetric Determination of Selenium</td>
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<tr>
<td>Primary Author</td>
<td>Jaime A. Pizarro</td>
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<tr>
<td>Co-Authors</td>
<td>Alan B. Placencia, Karina J. Diaz, María P. Oyarzún, Rodrigo A. Segura</td>
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<tr>
<td><strong>Abstract Text</strong></td>
<td>This work consists in the development of electrochemical sensors for the determination of selenium using modified electrodes with gold nanoparticles generated by electrochemical (CV/AuNPs/eq) and chemical method (CV/AuNPs/sq) using the technique of anodic stripping voltammetry (ASV) with sweep square wave voltammetry (SWV). CV/AuNPs/eq was characterized by cyclic voltammetry (CV), atomic force microscopy (AFM), scanning electron microscopy (SEM) and spectrometry X-ray scattering (EDS) finding a homogeneous distribution of gold nanostructures with a size of 75 nm. Chemical synthesis of gold nanoparticles was characterized by transmission electron microscopy (TEM) and UV-vis spectroscopy (surface plasmon resonance) obtaining spherical gold nanoparticles with an approximate size of 5 nm. CV/AuNPs/sq was characterized by cyclic voltammetry (CV). Under optimal conditions of accumulation potential (Eacc) -0.80 V, accumulation time (tacc) 120 s and 15 Hz frequency, validation was developed. CV/AuNPs/eq presented a linear range between 10 to 50 µg L-1 with a limit of detection (LD) of 0.120 µg L-1 and the linear range for CV/AuNPs/sq was 5-55 µg L-1 with an LD of 0.183 µg L-1.</td>
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<td><strong>Keywords</strong></td>
<td>Chemically Modified Electrodes, Electrochemistry, Stripping Analysis, Trace Analysis</td>
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Electrochemistry

One of the major problems of today’s world is in its heavy dependency on fossil fuels for energy requirements. So the increase uses of sustainable energy resources are very important. Hydrogen, the most abundant element on earth is the cleanest and ideal fuel (carrier), has been considered as the fuel of the future. The simplest way of hydrogen production is the electrolysis of water. In this sense, a wide variety of metal electrodes have been modified by conducting polymers which exhibit good catalytic activity in several electrochemical processes.

Chitosan has long been marked as one of the most promising natural polymers, has chemical inertness, high mechanical strength, biodegradability, biocompatibility, high-quality film-forming properties, and low cost. For this purpose, chitosan-assisted synthesis of conductive polymers has been extensively studied in recent years.

This study is focuses on the development of new forms of conducting polymer/metal nanoparticle composite materials as low-cost, efficient catalysts for hydrogen production from water. For this purpose, the electropolymerization of aniline was achieved on low cost graphite pencil electrode in the presence of chitosan in electrolyte medium. Conducive and mechanically strength composite films were obtained by incorporating chitosan to the polymer chains. Then the platinum (Pt) particles were electrodeposited on the composite on nano scale which has exhibit high catalytic activity on hydrogen production. The catalytic activities of all composite materials were investigated for electrocatalytic hydrogen evolution reaction. The electrocatalytic activities of the composite electrodes were studied by cyclic voltammetry, Tafel polarization curves, electrochemical impedance spectroscopy. The structure and morphology of the electrode materials were also investigated.

Acknowledgement
This research has been supported by the The Scientific and Technological Research Council of Turkey (TUBITAK-114Z315).

Keywords: Electrochemistry, Fuels\Energy\Petrochemical, Nanotechnology, Polymers & Plastics
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Electrochemistry
Spectroelectrochemistry is a well-known technique used to achieve concurrent multimode selectivity in one sensor. The sensor usually consists of an optically transparent electrode (OTE) coated with a charge selective polymer film. These polymer films are employed to pre-concentrate analyte at the OTE surface so that it can be sufficiently detected optically through electrochemical modulation, while lowering the detection limit. OTEs such as Indium Tin Oxide (ITO) have been used extensively in this method but little is known about the applicability of such sensors using other OTE materials, such as Boron Doped Diamond (BDD). We have developed an attenuated total reflectance (ATR) absorbance and fluorescence-based sensor with a BDD OTE coated with sulfonated ionomer films, Nafion and SSEBS. This is demonstrated using tris(2,2'-bipyridyl)ruthenium(II) ion [Ru(bpy)32+] and the results are compared to those obtained on ITO. One distinct advantage of BDD OTEs over ITO OTEs is their significant increase in sensitivity for organic compounds, such as para-aminophenol and hydroquinone. Using this sensor we were able to manifest new applications to broaden the use of spectroelectrochemistry and BDD as an electrode material.
For feedback controlled monitoring of the popular anesthetic, propofol, we used a voltammetric sensor coated with a highly-plasticized polymer membrane [1]. The sensitivity of the sensor is controlled by diffusion coefficient (D) of propofol in the membrane, while the detection limit and the selectivity depends on the partition coefficients (P) of the analyte and interfering compounds. Both D and P are strongly influenced by the composition of the membrane. To optimize the membrane composition for sub-micromolar detection limit and adequate selectivity, the diffusion and partition coefficients have to be measured in a variety of membranes.

In our contribution, we show that both D and P can be determined voltammetrically using planar electrochemical cell with a carbon fiber microelectrode. To show the feasibility of the method, first we determined the diffusion coefficients in a variety of organic solvents (e.g. tetrahydrofuran, dichloromethane) and plasticizers (e.g. octanol, ortho-nitrophenol octylether), both with and without supporting electrolyte. Diffusion coefficients were calculated from the steady state current recorded in solutions of electrochemically active compounds prepared in the different solvents. Once the diffusion coefficients were known, the steady state current measured in water-immiscible plasticizers -- following a liquid-liquid extraction -- could be used to calculate the organic/aqueous partition coefficients.

By coating the cell with a membrane, the same method could be used for determining D and P in the membrane. In this contribution, we report the diffusion and partition coefficient of a variety of compounds (e.g., ferrocene derivatives, propofol, p-acetamido phenol) in organic solvents/plasticizers and membranes.


Keywords: Biomedical, Electrochemistry, Extraction, Polymers & Plastics
Application Code: Biomedical
Methodology Code: Electrochemistry
Abstract Text
The spectroelectrochemical characterization of an optically transparent electrode (OTE) micro-fabricated sensor is investigated. The stability of the microsensor was improved by electroplating silver and chemically oxidizing silver chloride onto the platinum reference electrode. The sensor combines three modes of selectivity (electrochemistry, selective partitioning, and spectroscopy). The selective partitioning component of the sensor consists of a charge-selective polymer thin-film for preconcentration of the model compound. The spectroscopy component of the sensor is investigated using luminescence modulation. The use of the OTE fabricated microsensor with these three modes enhances the sensitivity of the sensor as well as selectivity for detection of trace concentrations of a target molecule.

Keywords: Electrochemistry, Microelectrode, Sensors, Spectroelectrochemistry
Application Code: General Interest
Methodology Code: Sensors
Restriction endonucleases are the main tool of modern genetic engineering. They are essential in recombinant technology, sequencing of a large DNA strands, genotyping, mapping, and also they play a crucial role in a molecular diagnostics. Because of their biological importance and wide use, a number of methods have been developed to assay restriction enzyme activity. However, all these protocols are time and DNA consuming, laborious, and usually require isotope or fluorophore labeling. Thus, the search for a new, simple and economical methods for endonuclease activity determination is ongoing.

In our work we have focused on monitoring enzymatic reaction using electrochemical biosensor and square wave voltammetry (SWV). A PvuII restriction enzyme and a 20-mer oligonucleotide dsDNA self-assembly monolayer (SAM) on gold, containing a restriction recognition site of a chosen enzyme, were used as a model system. The measurement of enzyme activity is based on decrease of current signal derived from the redox active probe–methylene blue (MB) present in the solution. The impact of DTT, BSA, TRITON X-100, and glycerol that are commonly used in enzymes solution on dsDNA-SAM was examined using electrochemical impedance spectroscopy (ESI). The influence of the distance of the restriction site from the electrode surface on the assay sensitivity was also evaluated. The kinetics of the enzymatic reaction using developed sensors with various oligonucleotide sequences will be presented as well.

This work has been supported by the European Union in the framework of the Regional Development Fund through the Joint UW and WUT International PhD Program of Foundation for Polish Science (Grant MPD/2010/4)
### Abstract Text

The electrospinning carbon fibers made from polyacrylonitrile (PAN) have attracted attention due to their easy processing, high carbon yield and robust mechanical properties. In this work, a glassy carbon electrode modified with carbon fibers was developed for the simultaneous determination of trace levels of lead and cadmium by anodic stripping voltammetry (ASV) (Figure 1). Compared to the bare glassy carbon electrode, the carbon fiber modified electrode greatly improves the sensitivity for cadmium and lead. The electrode was demonstrated with analysis of real samples. The interactions between lead and cadmium was investigated in detail.

### Keywords:
- Electrochemistry
- Electrodes
- Environmental/Biological Samples
- Raman

### Application Code:
- Environmental

### Methodology Code:
- Electrochemistry
Oxygen reduction reaction (ORR) is of interest in the areas of amperometric oxygen sensing, fuel cell, and etc. Since the ORR is a sluggish reaction requiring a high overpotential, relevant electrocatalysts are employed. Most commonly used catalyst, Pt, however, has the limitations of high cost, scarcity of supply, and susceptibility to poisoning by alcohol fuel. In our previous studies, we investigated the electrocatalytic activity of silver halide compounds for the ORR of fuel cells.

In continuation to our previous work, we performed first-principles density functional theory (DFT) calculations to investigate the reaction pathway of ORR on the 100 surfaces of electrocatalysts composed of metal halide (MX, X = Cl or Br). The catalytic mechanism of these electrocatalysts for ORR was elucidated by calculating the binding energies of ORR intermediates (e.g., O\(_2\), O, OH, OOH, HOOH, and H\(_2\)O) on the catalysts.

Our theoretical study provides a possible explanation for the experimental findings of 4 electrons or 2 electrons reduction paths on the metal halide electrocatalysts in comparison to pure Pt and pure metal electrocatalysts.

This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Ministry of Education, Science and Technology (NRF-2014R1A2A2A05003769).
Next generation techniques for bio-manufacturing, medical diagnostics and environmental monitoring demand high performance and low-cost chemical sensors. Electrochemical sensors are poised to significantly impact these technologies, because of their relatively low cost, simple construction, and ease of use. Unfortunately, the broad range of electrochemical sensors demonstrated by researchers has produced only limited commercial technologies. These limitations are partly due to the complexity of real-world samples and the inhomogeneity of sensing environments. Active control of an electrode’s temperature is one potential route to overcoming these challenges by both delivering a stable and known sensing temperature across measurements and providing an additional tunable experimental variable for improving measurement sensitivity and selectivity.[1,2]

Here we present the design, fabrication, and temperature characterization of microfabricated, temperature-controlled electrodes. The use of microfabrication provides several advantages by improving sensor reproducibility, control, and throughput. Heated disk electrodes with rapid thermal responses and independently controlled temperatures were fabricated with underlying Pt microheaters (Figure 1(a)). Fluorescent temperature imaging techniques were used to evaluate temperature gradients and thermal response times and their impact on sensor design and performance. Electrochemical measurements were performed on model systems (e.g. Fe(CN)₆³⁻/⁴⁻) in which coupled heat and mass transport impart signal enhancement, as shown in the cyclic voltammograms in Figure 1(b). We have also explored the impact of various temperature control routines, such as temperature steps, pulses and oscillations, on electrochemical signal content.

References:

Keywords: Biosensors, Electrochemistry, Electrodes, Temperature
Application Code: General Interest
Methodology Code: Electrochemistry
We have developed a new method to characterize the neurotransmitters bound to the dense core protein nanoparticles. We measure current transients from single vesicles impacting an electrode. When these transients are analyzed, 70% of the decay curves can be fit to a double exponential curve suggesting that the dense core inside the vesicle plays a role in the response. In addition, we are looking for collision events for dense core proteins alone as they impact the surface of a microelectrode. The transmitter molecules in the dense core protein should be oxidized at the electrode surface leading to a current that can be quantified by Faraday’s law. Dense core protein located in secretory vesicles is composed of charged polypeptides from the Chromogranin family. These can be isolated from chromaffin vesicles by density gradient centrifuge. This protein matrix accumulates and stores high concentrations of neurotransmitters, Ca2+ and ATP within the secretory vesicle. However, the mechanisms of accumulation and release of neurotransmitter molecules are unknown and highly debated. The aim of this project is to study how the protein matrix in large dense core vesicles affects the storage and regulation of neurotransmitters both in the dense core and in the nano-environment of each vesicle.
Electrochemistry Mediated Desalination

Electrochemically mediated desalination is an energy efficient and membraneless method to separate salts from water by using electrochemistry. The process relies on the generation of a local electric field gradient near an electrode embedded at the junction of a bifurcated microchannel structure. At the electrode, a small percentage of the naturally abundant chloride in seawater is oxidized to chlorine, consequently producing a localized ion depletion zone and electric field gradient that increases ion electromigration, therefore separating ions to produce partially desalted water. Moreover, we describe how applied potential bias, chloride concentration, flow rate, and electroactive surface area affect the desalination process.

Keywords: Electrochemistry, Electrophoresis, Lab-on-a-Chip/Microfluidics, Water
Application Code: General Interest
Methodology Code: Electrochemistry
Today, ion-selective electrodes (ISEs) are capable of detecting nanomolar concentrations of metal ions. However, ISEs require a long pre-conditioning step in a solution containing the analyte ion. During that period, a zero-current ion flux is established while the ionophore is saturated with the analyte ion. This required step highly limits ISEs’ in-field application. Here, we present ISEs for Na\(^{+}\), Ag\(^{+}\), and I\(^{-}\) with Nernstian response and submicromolar limits of detection that do not require pre-conditioning step.

Na\(^{+}\)-ISE and Ag\(^{+}\)-ISE contain 10 mmol kg\(^{-1}\) of sodium ionophore X and copper (II) ionophore I, respectively, and 5 mmol kg\(^{-1}\) of sodium tetrakis[3,5 bis(trifluoro-methyl) phenyl] as ion-exchanger. I\(^{-}\)-ISE contained 1 mmol kg\(^{-1}\) mercuracarborand-3 and 0.75 mmol kg\(^{-1}\) of tridodecylmethylammonium chloride. All ISEs were prepared using a PVC/DOS matrix. Further, 25 \(\mu\)L of 0.1 M AgNO\(_3\) and 25 \(\mu\)L of 0.1 M NaI was added directly to the Ag\(^{+}\)-selective and I\(^{-}\)-selective cocktails, respectively. No analyte ion solution was added to the Na\(^{+}\)-ISEs. Potentiometric measurements were conducted without any conditioning time using a high-input impedance EMF-16 multichannel data acquisition system. The results obtained indicate that introduction of analyte ion directly to the ion-selective cocktail allows the ionophore to chelate with the analyte ion and execute the ion-exchange process solely within the membrane. Thus, establishing the zero-current ion flux at that step without needing a pre-conditioning step.

The authors acknowledge the Office of Research & Commercialization, College of Sciences and the Department of Chemistry at the University of Central Florida for financial support.

Keywords: Electrochemistry, Electrodes, Potentiometry
Application Code: General Interest
Methodology Code: Electrochemistry
Rational Design of MMA-DMA Copolymer to Improve the Limit of Detection of Ion-Selective Electrodes

The goal of this project is to synthesize different ratios of methyl-methacrylate (MMA) to decyl-methacrylate (DMA) to create a matrix that will allow improvement of the limit of detection of Ion-Selective Electrodes (ISEs). Usually ISEs are prepared using poly(vinyl chloride) (PVC) plastisized with bis(2-ethylhexyl sebacate) (DOS) as matrix support. PVC-DOS is the most common choice since it is commercially available and has a coefficient of diffusion of $\sim 10^{\text{-8}} \text{cm}^2\text{s}^{-1}$. However, DOS can leach out of the membrane matrix with time prejudicing the performance of the electrode. Recently, the copolymer MMA-DMA, which has a coefficient of diffusion of $\sim 10^{\text{-11}} \text{cm}^2\text{s}^{-1}$, has shown to allow low limits of detection in the nanomolar range when utilized in membranes for ISEs. Thus solid contact ISEs were created by coating a gold wire with a polymer conductor (poly-3-octylthiophene), a polymeric membrane that contains an ionophore sensitive to a particular ion of choice, an ion exchanger [3,5-bis(trifluoromethyl) phenyl]borate, as well as the copolymer MMA-DMA. The limit of detection was evaluated with different ratios of MMA-DMA and the best limit of detection was found when the ratio of 41.5-58.5 was used. The authors acknowledge the College of Sciences and Department of Chemistry at the University of Central Florida for financial support of this research.

Keywords: Analysis, Electrochemistry, Ion Selective Electrodes, Membrane
Application Code: General Interest
Methodology Code: Electrochemistry
Electrochemistry

In Situ Electrochemical Synthesis of Zinc Substituted Hydrotalcite Films for Corrosion Protection

Stainless steel is a versatile material used in the oil & gas, construction, mining, pharmaceutical, and food/beverage industries. Stainless steel is resistant to corrosion because of the passive oxide layer that forms as a result of its chromium component. However, this oxide layer is destroyed faster than it is created in low-oxygen and poor circulation marine environments. A synthetic hydrotalcite film can protect the stainless steel surface from attack by chloride ions. Hydrotalcite is a layered material which contains alternating positively charged metal hydroxide layers and negatively charged layers composed of anions and water. The anions in the negatively charged layer may be exchanged. This anion exchange property could allow the synthetic hydrotalcite film to trap Cl- ions that would otherwise destroy the protective oxide layer. The film can be deposited electrochemically which allows greater control over thickness and deposition over irregular substrate geometries. Electrodeposition is also cheaper than most other film deposition techniques. Hydrotalcite is a naturally occurring clay in its Mg-Al form. In this study a zinc substituted film will be generated in situ at the electrode by the reduction of nitrate resulting in hydroxide formation. The electrodeposited synthetic hydrotalcite structure was confirmed by FTIR and XRD. Film morphology was observed by SEM and improvements in corrosion resistance were supported by linear polarization after various immersion times.

Abstract Text
Stainless steel is a versatile material used in the oil & gas, construction, mining, pharmaceutical, and food/beverage industries. Stainless steel is resistant to corrosion because of the passive oxide layer that forms as a result of its chromium component. However, this oxide layer is destroyed faster than it is created in low-oxygen and poor circulation marine environments. A synthetic hydrotalcite film can protect the stainless steel surface from attack by chloride ions. Hydrotalcite is a layered material which contains alternating positively charged metal hydroxide layers and negatively charged layers composed of anions and water. The anions in the negatively charged layer may be exchanged. This anion exchange property could allow the synthetic hydrotalcite film to trap Cl- ions that would otherwise destroy the protective oxide layer. The film can be deposited electrochemically which allows greater control over thickness and deposition over irregular substrate geometries. Electrodeposition is also cheaper than most other film deposition techniques. Hydrotalcite is a naturally occurring clay in its Mg-Al form. In this study a zinc substituted film will be generated in situ at the electrode by the reduction of nitrate resulting in hydroxide formation. The electrodeposited synthetic hydrotalcite structure was confirmed by FTIR and XRD. Film morphology was observed by SEM and improvements in corrosion resistance were supported by linear polarization after various immersion times.

Keywords: Electrochemistry, Materials Characterization
Application Code: Other
Methodology Code: Electrochemistry
Fast-scan cyclic voltammetry (FSCV) with carbon-fiber microelectrodes is a popular technique for \textit{in vivo} and \textit{in vitro} detection of various electroactive species including catecholamines. FSCV is utilized within several different fields of science including chemistry, biochemistry, neuroscience, and psychology. Inconsistencies in carbon-fiber microelectrode calibration methodology have become apparent because of its popularity within different disciplines. Proper calibration of carbon-fiber microelectrodes is essential so that faradaic current measured \textit{in vivo} can be related back to appropriate concentration values. Calibrations are performed in buffers that mimic the extracellular fluid of the brain, but previous work has shown the response of the carbon-fiber microelectrode strongly depends on buffer composition. Currently there are several buffers commonly used for electrode calibration including phosphate-buffered saline (PBS), tris(hydroxymethyl)aminomethane (Tris), and a carbonate-based artificial cerebral spinal fluid (aCSF). Here the response of carbon-fiber microelectrodes was characterized for a variety of analytes in these buffer systems including dopamine, pH change, oxygen, serotonin, norepinephrine, and epinephrine. For each analyte in each buffer, limit of detection, sensitivity, 10%-90% rise time, and signal to noise ratio were quantified. These results will be helpful in improving electrode calibrations and comparing results obtained under a variety of working conditions.
**Abstract Text**

It is highly desirable to develop sensitive and simple methods for detection of lead ion since lead ion is a toxic metal ion due to its causing a number of adverse health effects. A novel label-free electrogenerated chemiluminescence (ECL) biosensor for the determination of lead ion was developed on the basis of DNAzyme consisting DNAzyme strand (5E) and substrate strand (5DS) with a ribonucleotide adenosine served as molecular recognition element and ruthenium(II)-(1,10-phenanthroline) (Ru1) as an intercalation ECL signal compound. The ECL biosensor was fabricated by coupling lead ion-specific GR-5 DNAzyme, 5\text{-}\text{ethynyl}-\text{(CH2)}_4-5E-3, 5\text{-C-C-(CH2)}_4-\text{ACA GAC ATC ATC TCT GAA GTA GCG CCG CCG TAT AGT GAG-3 (5E)} to 4-azidoaniline modified glassy carbon electrode using “click chemistry”, and then hybridizing 5DS (5\text{-}CTC ACT AT rA GGA AGA GAT GAT GTC TGT-3) with the 5E•DS to form a double-stranded DNA (5E•DS) and further intercalating Ru1 into the 5E•DS. Upon binding of lead ion to the 5E•DS, the formed complex catalyzed the cleavage of the 5DS at the site of the rA, and the 5E•DS was dissociated and Ru1 was released, led to decrease ECL signal. In this paper, electrochemical “click chemistry” immobilization of GR-5 DNAzyme was developed. The ECL intercalator, the concentration of Ru1 and the incubation time were optimized. The results showed that the decreased ECL signals were directly proportional to the concentrations of lead ion in the range from 20 pM to 1.0 nM with an extremely low detection limit of 7.2 pM lead ion. The biosensor showed a high reproducibility and long-term storage stability. The biosensor was successfully applied to determination of lead ion contents in blood samples. This work demonstrates that using Ru1 intercalation and “click chemistry” for the fabrication of the ECL biosensor with high sensitivity and significant regeneration ability is promising approach for the design of label-free ECL biosensor for the determination of metal ions.

**Keywords:** Bioanalytical, Biosensors, Chemiluminescence, Electrochemistry

**Application Code:** Clinical/Toxicology

**Methodology Code:** Electrochemistry
Hydroquinone (HQ) and catechol (CC) are two important isomers of phenolic compounds, which are often used in cosmetics, pesticides, flavoring agents, antioxidant, secondary coloring matters, and photography chemicals. The absorption of HQ or CC from the gastrointestinal tract can induce some disease such as renal tube degeneration and liver function decrease. Because of their high toxicity and low degradability in the ecological environment, HQ, and CC are considered as environmental pollutants by the US Environmental Protection Agency and the European Union. A simply and high selectively electrochemical method for simultaneous determination of HQ and catechol (CC) has been developed at an electrochemically activated screen-printed carbon electrode (SPCE) modified with Prussian Blue (PB). The PB acted as a mediator and thereby enhanced the rate of electron transfer in chemical reaction. Various optimization studies such as the pH of the measuring solution, linear range of response, sensitivity and detection limit, were conducted to obtain maximum amperometric responses for analytes measurement. Under the optimized condition in DPV, the oxidation peak current of HQ and of CC is linear over a range from $4.0 \times 10^{-6}$ M to $9.0 \times 10^{-5}$ M HQ and from $1.0 \times 10^{-6}$ M to $9.0 \times 10^{-5}$ M CC. The obtained detection limit for HQ and CC was $1.17 \times 10^{-7}$ M and $4.28 \times 10^{-7}$ M, respectively. The proposed activated PB-SPCE also exhibited good selectivity and was successfully applied to the simultaneous determination of HQ and CC in spiked tap water.

Keywords: Chemically Modified Electrodes, Electrochemistry, Environmental, Voltammetry
Application Code: Environmental
Methodology Code: Electrochemistry
Fibrillation of proteins constituting biologic drugs is an undesirable property that can render these drugs useless in the treatment of patients. Fibrillation occurs when proteins become misfolded and aggregate.\(^\text{1}\) A variety of factors can cause fibril formation, such as agitation of protein solutions, increased temperatures in the surrounding environment, or simply a large timeframe between the time of drug manufacture and use.\(^\text{2}\) This study explores the development and application of a method to fluorescently-label insulin aggregates and fibrils with Nile Red in order to monitor the growth of these species using fluorescence correlation spectroscopy (FCS). The FCS method employs the determination of protein hydrodynamic diameter which is used in the study as the basis for kinetically analyzing insulin aggregation and its subsequent fibrillation. Studies are also in progress to compare and contrast the accuracy of the method’s determination of protein hydrodynamic diameter with the corresponding results obtained via dynamic light scattering (DLS). Future work will target expanding the method to other proteins capable of undergoing fibrillation.


A Turn-On Fluorescent Probe for Sodium Azide Detection

Sodium azide is used in agricultural, laboratory, and commercial purposes, and thus is a significant environmental concern. In addition, several poisoning incidents have been reported due to the toxicity of azide. A simple and selective inorganic azide detection method using fluorescence has been developed. Based on Strain-Promoted Azide–Alkyne Cycloaddition (SPAAC) mechanism, the probe’s fluorescence is turned on upon selective reaction with sodium azide. Quantitative analysis can be achieved in linear range of 2-100 µM.

Abstract Text

Sodium azide is used in agricultural, laboratory, and commercial purposes, and thus is a significant environmental concern. In addition, several poisoning incidents have been reported due to the toxicity of azide. A simple and selective inorganic azide detection method using fluorescence has been developed. Based on Strain-Promoted Azide–Alkyne Cycloaddition (SPAAC) mechanism, the probe’s fluorescence is turned on upon selective reaction with sodium azide. Quantitative analysis can be achieved in linear range of 2-100 µM.

Keywords: Detection, Fluorescence, Quantitative, Sensors
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
In the last years there has been a great interest towards the development of optical nanosensors. To this end, fluorescent nanomaterials have been implemented in analytical systems for the detection of toxic metal species. Recently, fluorescent carbon dots (CDs) have received much attention due to their attractive properties such as strong fluorescence, tunable color emission and high photostability. Mercury and its compounds are well-known environmental pollutants. Among mercury species, methylmercury is the most toxic to living systems due to its ability to cross biological membranes resulting in accumulation and bioamplification through the food chain. Since the main human exposure to this toxic is linked to fish consumption, the control of methylmercury levels has become of paramount importance. Conventional methods for detecting methylmercury are typically based on the use of complex hybrid techniques involving a chromatographic separation coupled to a specific detector. Further efforts should be focused on development of simple and fast assays with the possibility of on-site analysis.

In this work, a novel assay that integrates the synthesis of fluorescent CDs and sensing within a single step for methylmercury detection is presented. To this end, high-intensity sonication of carbohydrates is used for the synthesis of CDs in the presence of the target analyte. It has been observed that ultrasound facilitates the permeation of methylmercury through the passivation coating of CDs made of PEG, which causes the fluorescence quenching as a result of a non-radiative electron/hole recombination. This novel assay allows detecting methylmercury at nM level in only 1 min using very low amounts of organic precursors and a portable micro-fluorospectrometer.

Acknowledgements: Financial support from the Spanish Ministry of Economy and Competitiveness and the European Commission (FEDER) (Project CTQ2012-32788) is gratefully acknowledged.

Keywords: Fluorescence, Mercury, Nanotechnology, Sensors
Application Code: Nanotechnology
Methodology Code: Fluorescence/Luminescence
DNA-driven, homogenous assays for the quantitation of proteins are simple and easy to perform, yet there are still challenges to widespread acceptance for diagnostics due to non-optimal signal-to-noise (S/N) ratios. This problem stems from difficulty in optimizing assay conditions in the presence of background. In this study, we devised a high-throughput analytical tool, thermofluorimetric analysis (TFA) using a qPCR instrument, which allows separation of signal and background components from total readout in solution. This approach takes advantage of the different thermal stabilities of background components and signal components. Using this tool, we easily optimized the conditions for several homogenous fluorescence assays such as proximity fluorescence resonance energy transfer assay (pFRET) and proximity ligation assay (PLA). Non-amplified pFRET assays with TFA showed a limit of detection (LOD) of 6 picomoles of thrombin in mouse serum. Importantly, the direct fluorescence readout for pFRET, which is typically ineffective in autofluorescent serum samples, was made effective using this approach. Amplification of signal with PLA allowed further improvements in LOD, as low as 200 attomoles of thrombin in mouse serum samples. This work shows that TFA is not only a highly valuable tool in optimizing proximity immunoassays, but we also show that probe valency can be studied further and optimized with thermally resolved fluorescence readout.

Keywords: Bioanalytical, Fluorescence, Nucleic Acids, Protein
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
The development of new chemical-mechanical planarization (CMP) slurries designed for polishing microelectronic structures of decreasing linewidth has driven the demand for improved metrologies to characterize the abrasive component of emerging CMP slurries. Evaluation of particle size for small abrasive particles (hydrodynamic diameter < 20 nm) and the adsorption of slurry additives onto dispersed abrasive particles in a CMP slurry is an evergreen need which can be achieved using a single-molecule spectroscopic method, fluorescence correlation spectroscopy (FCS).

FCS employs a confocal fluorescence microscope to both excite and collect the fluorescence emission from abrasive particles rendered fluorescent by the adsorption of fluorescent dyes. In this presentation the application of FCS for the characterization of the smallest particles in the particle size distribution (PSD) of silica abrasive particle dispersions used in CMP slurries is described. PSDs are determined from FCS data for the dispersions by employing algorithms capable of analyzing the polydispersity of the distributions. To further evaluate the method’s capability, the PSDs of silica dispersions were compared before and after fractionation by ultracentrifugation. This analysis demonstrated the enhanced sensitivity of FCS for analyzing smaller particle fractions relative to dynamic light scattering determinations of the same particle fractions. These data better defined the experimental FCS conditions needed for accurate PSD analysis and enabled the determination of Langmuir adsorption isotherms for dye binding to silica abrasive particles.

This research has been generously funded by Intel Corporation.
In our work we proposed a novel approach to quantification relatively small amounts of water present in low polarity, aprotic solvents using fluorometry. This approach takes advantage of protolitic reaction of fluorophore : 4-methylumbelliferone dissolved in the solvent with water, acting as a base. The low emission intensity neutral 4-methylumbelliferone is transformed in reaction with water to a highly fluorescent anionic form. Thus the increase in emission intensity is observed for increasing water contents in aprotic solvents. It is shown that organic impurities of protolitic character (amines, acids) not only do not interfere with water contents determination but increase the sensitivity of emission vs. water contents dependence. Using fluorescence more of measurements eliminates the disadvantages resulting from turbidity of the sample, which may be a common phenomenon in industrial solvents. For low water contents and highly lipophilic solvents this method yields (in practical conditions) higher sensitivity compared to Karl Fischer titration.
Fluorescence/Luminescence Bioanalytical and Other Applications

Abstract Title
A Competitive Approach in Investigating the Binding Interactions of Mangiferin with Human Serum Albumin

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Abstract Text
Mangiferin is a natural polyphenol glucosylxanthone obtained from the mango tree, honey bush and other sources. The clinical applications of mangiferin are well known. It is commonly used during the diagnosing of dysentery, infection, inflammation, allergies, cancer and diabetes. Studying the interaction between mangiferin and human serum albumin (HSA) is important as it provides an indirect insight into the transportation and the distribution of the ligand with the carrier protein. Furthermore, any binding results obtained could be useful for elucidating ligand-carrier protein binding patterns.

In part, the study of mangiferin is useful in medicine; however, its structure and binding affinity make it potentially useful as a binding agent. Based on the given information, a proposed specific use of mangiferin is as a competitive binding agent to the serum albumin. However, given it is likely to compete with other common HSA binding ligands such as cyanine dyes, any change to the absorbance or emission properties of the latter can easily serve as a tool to monitor ligand binding.

Our initial studies have revealed that Human serum albumin exhibit high binding affinity for mangiferin similar to the water-soluble cyanine fluorophores, which interacts at the subdomain IIA and IIIA. It is also believed that the Mangiferin binds to the hydrophobic binding region of subdomain IIA, where the tryptophan residue, trp-214, is present. This binding is proposed to be due to specific hydrophobic, H-bonding effects and other non-covalent interactions. Considering there is limited research data available on this binding relationship, our competitive studies are quite useful in fully understanding the causation for high binding affinity to human serum albumin and further explaining this rather complex binding equilibrium.

Keywords: Bioanalytical, Biopharmaceutical, Fluorescence, UV-VIS Absorbance/Luminescence
Application Code: Biomedical
Methodology Code: Fluorescence/Luminescence
Bacterial endotoxin is a component of gram-negative bacterial cell walls and causes fever or shock when it enters the human blood stream. Therefore, the concentration of bacterial endotoxin in pharmaceutical and dialysis solutions needs to be strictly controlled. The conventional methods for the detection of bacterial endotoxins use reactions with limulus amebocyte lysate (LAL) that lead to gel-formation, a change of turbidity, or a change of color. Since of the problems including the long measuring time and the low sensitivity in the conventional methods, the novel method performing in more rapid and higher has been required. Here, we report a novel endotoxin detection method based on a combination of the limulus reaction and bioluminescence using mutant firefly luciferase. Our method gave higher sensitivity for bacterial endotoxins in a shorter time. However, it also had the problems of stability and reproducibility introduced by the unstable reagents.

In this study, lyophilizations for all of the reagents were studied to achieve good performance, including both long-term stability and good reproducibility. The lyophilized reagents allowed the optimization of both the concentration of LAL and the lyophilize condition, the bacterial endotoxin at a concentration of 0.001–0.01 EU/mL was detected within 20 minutes. The correlation coefficient was >0.99 and the relative standard deviation was <15%. A good correlation for the conventional method was also observed. The correlation coefficient for the turbidimetric method was <0.86. The concentration of bacterial endotoxin in pharmaceutical and dialysis solutions can be measured easily, rapidly, and at a high sensitivity by our method.
The primary goals of disinfectants are to kill bacteria and inactivate viruses. However, disinfectants can also react with many other chemical matters present in water, forming potentially toxic disinfection by-products (DBPs) that can be found in finished drinking water. And because of the widespread use of chlorination, the two major prominent DBPs in chlorinated drinking water are trihalomethanes (THMs) and haloacetic acids (HAAs), which are regulated by the United States Environmental Protection Agency (USEPA). It is well investigated that THMs and HAAs are formed from both disinfectants and natural organic matter (NOM). However, to date several hundred and non-regulated DBPs can be found in drinking water. These DBPs other than the regulated THMs and HAAs are known as emerging disinfection by-products (eDBPs) and may be more toxic than current regulated DBPs.

In our laboratory, a preliminary kinetic spectrophotometric method was performed to investigate the reaction and use of dimethylamino pyridine (DMAP) as a derivatization reagent to analyze HAAs and three classes of emerging disinfection by-products (eDBPs) in drinking water, including haloacetamide, haloacetaldehyde and haloacetonitrile.

The research will focus on expanding the use of DMAP to derivatize two additional classes of eDBPs, which are halomethanes, and halonitromethanes. Absorbance and fluorescence of the reaction between DMAP and each of the five classes of eDBPs along with HAAs will be measured. Additionally, a structural identification of the reaction products using mass spectrometry (MS) will be presented to understand the reaction mechanism between DMAP and eDBPs. This research will provide information in attempt to develop reliable spectrophotometric method for the determination of eDBPs in drinking water.

Keywords: Chromatography, Derivatization, Environmental/Water, Fluorescence
Application Code: Environmental
Methodology Code: Fluorescence/Luminescence
Although alkylammonium formate (AAF) ionic liquids are quite viscous and are thought to form micelle type structures, the potential enhancement of fluorescence by fused ring organic compounds using AAF solvents has not been studied in detail. We have compared two ionic liquids, ethylammonium formate (EAF) and isopropylammonium formate (IPAF), as solvents for the fluorescent determination of pharmaceuticals. Solid phase extraction of these AAF solvents using activated carbon or a polystyrene-pyrrolidinone polymer has been shown important to reduce the excitation background, particularly at 280 nm as compared to 350 nm. AS the % EAF in water was varied from 10-90%, the fluorescence of riboflavin and naproxen remained quite constant until decreasing slightly at 80 and 90% EAF. However, over the same solvent range, warfarin showed a marked enhancement of fluorescence, more than double. Varying the % IPAF over the same range, riboflavin, in contrast, showed an increase in fluorescence of about 5 times from 10% to 60% before decreasing at higher % IPAF levels. Although not the case for EAF, tryptophan showed enhanced fluorescence in a 90% IPAF-10% water solution as compared to just an aqueous solvent. The fluorescence of other indoles such as serotonin, indole-3-acetic acid, and melatonin in varying percentages of IPAF is under study. The higher viscosity of IPAF and likely better micelle formation both seem to be contributing factors for the more pronounced solvent based fluorescence enhancement as compared to that for EAF.
### Abstract Text

The Abraham general solvation model can be used to predict partition coefficients for many compounds. This model describes and predicts the chemical properties of drugs with biological barriers. Solute properties are calculated using logarithmic analysis of both gas and liquid chromatographic retention times, which predict molecular descriptors of new and untested drugs. Our research used six gas chromatography columns and six liquid chromatography columns to develop accurate predictions. Retention times of drugs were measured and the solute properties were calculated. Using the molecular descriptors found in literature, the Abraham model was used to predict process coefficients. For each of the columns used, a correlation equation was developed for predicting descriptors. A standardized data process has been created in order to easily and quickly obtain molecular descriptors for new designer or illicit and illegal drugs. In order to get the descriptors, a drug must simply be run on the columns and retention times collected. Once collected, the retention times are compiled into the correlation equations and the molecular descriptors are calculated.

### Keywords:

- Drugs
- Gas Chromatography
- Liquid Chromatography

### Application Code:

- Bioanalytical

### Methodology Code:

- Liquid Chromatography
Various steroids, such as dexamethasone, betamethasone, cortisone, hydrocortisone, and prednisolone, are commonly used as anti-inflammatory medications. Separation of mixtures of these steroids is challenging due to similarity in their chemical structure. Here, we describe the development of reversed-phase chromatographic methods for separation of these steroids. Particular interest is paid to chemical differences between solute pairs, such as the presence or absence of carbon-carbon double bonds, the presence or absence of hydrogen-bonding ability, and the presence of carbon-fluorine bonds. Due to the minor difference in structure between solute molecules, highly selective chromatographic systems are required. Of particular interest is the use of stationary phases which contain specific functionality, such as embedded-polar-group phases or fluorinated phases. In addition, biologically-inspired stationary phases, such as those containing a cholesterol or membrane structure, are examined. In combination with these selective stationary phases, we employ mobile phases with selective additives. Derivatized beta-cyclodextrins have been shown to form strong complexes with some steroids, and here that ability is used to vary chromatographic selectivity. The effect of other variables, such as mobile phase modifier choice (methanol vs. acetonitrile) and temperature are also considered.
When conducting impurity analysis of pharmaceuticals, researchers need to detect trace level impurities along with the main analyte, which is present at a high concentration. To accomplish this, a detector that provides linearity over a wide concentration range is required. Furthermore, a high S/N (signal-to-noise ratio) is also required of the detector in order to provide high-accuracy quantitation of any impurities present at trace levels. Here, we introduce our evaluation of the sensitivity and linearity obtained in impurity analysis using the new SPD-M30A photodiode array detector with the optional high sensitivity cell (optical path length 85mm). To compare its performance with that of the former model (with 5mm path length cell), 2 µL of a 0.02 mg/L ketoprofen standard solution was injected using the same analytical conditions, and the respective S/N values were verified. Comparison of the results indicate that the S/N for the SPD-M30A with the high sensitivity cell is more than 10 times greater than that of the peak for the former model, demonstrating excellent quantitative performance. To check the linearity of ketoprofen detection using the SPD-M30A with the high-sensitivity flow cell, we conducted measurements using a sample concentration range of 0.01 – 500 mg/L. Excellent linearity was obtained in a range of 0.01-50 mg/L, with a correlation coefficient (R2) greater than 0.999. Since the linearity was not maintained when the concentration exceeded 50 mg/L, we used the Intelligent Dynamic Range Extension Calculator (i-DReC) function in LabSolutions software to extend the range of the calibration curve to 500 mg/L. With this function, the correlation coefficient (R2) improved to better than 0.999, and the error associated with each calibration point improved to less than 5%.
Approximately 50% of all drug molecules used in pharmaceutical products are reported to be ionic compounds. Ion chromatography is generally appropriate to analyze inorganic or organic ions, but not suitable for active pharmaceutical ingredients (APIs) analysis due to their hydrophobicity. On the other hand, reversed phase liquid chromatography (RPLC) is mainly applied for analysis of APIs, but cannot retain commonly-used ions for drugs. Consequently, it is difficult to analyze APIs and their counter-ions simultaneously.

In this study, we evaluated the ReDual column, our newly-developed mixed-mode column, for simultaneous analysis of APIs and their counter-ions. Mixed-mode column usage has increased because of the ability to analyze a wide range of compounds in a single run by multimode retention mechanisms. The ReDual column was able to analyze APIs and their counter-ions simultaneously. In addition, we investigated how retention behavior changes with parameters such as pH, ion strength, and concentration of organic solvents in the mobile phase.

Keywords: Liquid Chromatography, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
HPLC-based lipophilicity methods most often employ silica-based C18 columns for ballpark octanol-water logP estimates. Transcellular permeability, however, is better modeled by alkane-water logP. Despite growing interest in logP\(_{\text{alk}}\) this parameter is underutilized due to various experimental barriers and lack of robust high-throughput platforms for measurement.

The goal of this study was to evaluate a C18-functionalized PS-DVB-based column for its capacity to provide logP\(_{\text{alk}}\) estimates for a diverse set of solute and drug compounds when operated in fast gradient mode. Linear solvation energy relationship (LSER) analysis was used to characterize this system and to provide a meaningful basis from which the balance of intermolecular forces could be compared with those of other chromatographic and partition
Method validation is an important regulatory requirement for pharmaceutical organizations to demonstrate that a method is suitable for its intended purpose. A compliant laboratory must provide documented evidence and assurance that the analytical method generates accurate and reliable results. As the validation is a complex and prone-to-errors process, a well-organized plan is essential for successful validation and to ensure that the appropriate regulations and guidelines are being followed.

Here, we will present validation of a UPLC method for metoclopramide HCl and related substances. The study was performed using Empower 3 Method Validation Manager (MVM) software. The entire validation workflow from creating a validation protocol to acquisition, reviewing and processing, calculations, approval, and final reporting of the validation results will be discussed. The MVM software tracks every step of the validation process, flagging any steps or data that do not meet the validation requirements. This ensures compliance to the validation requirements and acceptance criteria defined in the protocol. Validation calculations and statistics are performed directly within the MVM software.

In this study, we validated the UPLC method for linearity, detection and quantitation limits, accuracy, repeatability, intermediate precision, specificity, and robustness using MVM software. First, we created a validation protocol to define the validation requirements and the acceptance criteria for each validation test. Then, we executed the validation study. We will demonstrate that method specificity is enhanced using orthogonal detection, ACQUITY QDa mass detector in conjunction with UV, to track peak homogeneity for the API and its related substances.

Keywords: Data Analysis, Pharmaceutical, Validation
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
There are benefits to the use of smaller diameter columns. These include increased sensitivity, a reduction in the amount of sample required, and substantially decreased solvent consumption and waste generation. To reap these benefits, technical hurdles in solvent delivery, sample introduction and the management of extra-column dispersion must be surmounted. Recently, commercial systems have become available that can address these challenges. Optimized microscale system configurations enable the migration of chromatographic methods from the common, analytical scale 2.1 mm diameter columns to microscale columns with 1.0 mm and 300 µm diameters. The goal of such a transition must be a method that not only exhibits the enhancements mentioned, but also conserves the selectivity, resolution and reliable quantitation of the original. Effective method migration, then, will necessitate thoughtful adjustments to method and instrument parameters in order to preserve the analytical quality of the original chromatography. A systematic protocol for this process is needed. This work presents the results of investigations into solvent delivery, injection behavior, UV and MS detection, and column temperature effects observed in small molecule microscale separations. The results are presented in the context of their overall influence on the chromatographic measures of a successful method migration. The experimental observations, when combined, will serve as a protocol for method migration from the analytical scale to the microscale.

**Keywords:** Lab-on-a-Chip/Microfluidics, Liquid Chromatography, Liquid Chromatography/Mass Spectroscopy, Small Molecule Microscale Separations

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography
**Abstract Text**

Given the diverse selection of pesticides used globally with differing regulatory limits in various countries, multi-analyte screening strategies capable of efficiently detecting residue violations are required. The QuEChERS methods have simplified and streamlined sample preparation for pesticide analysis of many fruits and vegetables. In recent years the use of accurate mass instruments has been at the forefront of food and environmental screening discussions. This has also been reflected in the evolving SANCO and CODEX guidelines that promote the use of accurate mass technology to address the challenges faced by industry.

Such accurate mass technology should provide high resolution, without compromising system sensitivity. Another challenge often associated with non-target analysis is the time required for effective data processing. In this pesticide screening study, a new quadrupole-time of flight mass spectrometer system shows accurate detection of all pesticide residues, while maintaining high resolution in compliance with global regulations. Furthermore, a 3D peak detection algorithm system simplifies data processing, where by a single scientific information system performs data acquisition, efficient data processing and data management requirements. With access to an extensive scientific library a comprehensive workflow to screen, quantify, and confirm residues in a single solution is demonstrated as a simple and efficient procedure for data interrogation.

**Keywords:** Food Safety, Liquid Chromatography/Mass Spectroscopy, Pesticides, Software

**Application Code:** Food Safety

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Instrument design in mass spectrometry (MS) relies on the use of magnetic and electric fields for manipulating the trajectories of ions inside a mass spectrometer. For example, radio-frequency (RF) fields are commonly applied to quadrupoles, hexapoles, and octapoles to guide ions from ion generation sources to analyzers and/or detectors. Hence, RF power supplies are typically used with various MS types. In this presentation, we will discuss results from the applications of a novel power supply for generation of RF signals in the range of 100 KHz to 20 MHz (up to 400 Vp-p) using readily available and inexpensive off-the-shelf components. Moreover, we will present a method for designing programmable control modules using common hobby-grade microcontroller electronics that have not previously been explored for use in MS instrument designs.

The instrument module consists of a miniature (< 1 inch³) variable frequency oscillator, a small (3”x7”x1”) commercial RF amplifier, and a home-built air core transformer. The programmable “brain” of the module was developed using heavily documented, open source, and inexpensive microcontrollers. Application of these design techniques can lead to potentially cheaper and faster mass spectrometer instrument prototyping.

Results from mass spectral analysis using the recently developed radio-frequency ionization (RFI) technique [1] coupled to a Fourier transform-ion cyclotron resonance (FT-ICR) MS using an RF signal of 12.10 MHz and 380 Vp-p will be reported. In addition, novel applications of this new RF generator, such as ion isolation and ejection in FT-ICR MS, will be discussed.


Keywords: Automation, Ion Cyclotron Resonance, Tandem Mass Spec
Application Code: Other
Methodology Code: Mass Spectrometry
Two-dimensional separations are useful to separate complex mixtures. Most techniques, however, require multiple steps. Pressurized simultaneous chromatography and electrophoresis is a novel technique that produces two-dimensional separations of complex mixtures in a single step. In addition, two mixtures may be spotted opposite each other onto a single pre-wetted TLC plate and separated concurrently for comparison. The TLC plate is pressurized between two acrylic plates while mobile phase is forced through the plate via syringe pump. An electric field is applied across the wetted plate orthogonal to the flow of mobile phase. Mixtures are separated according to their polarity and charge-to-mass ratio by chromatography and electrophoresis, respectively. By controlling pressure and mobile phase flow, separation occurs in as few as 4 min. A range of flow rates and electric potentials were tested to find and report the conditions that lead to the lowest plate height. Pressurized simultaneous chromatography and electrophoresis offers an effective, inexpensive, and fast alternative over other two-dimensional separation techniques.

**Keywords:** Chromatography, Electrophoresis, Separation Sciences, Thin Layer Chromatography

**Application Code:** General Interest

**Methodology Code:** Separation Sciences
Ionic liquids (ILs) are semi-organic compounds with attractive physical and chemical properties such as negligible vapor pressure, low melting points and feasible miscibility within a range of solvents. A key factor of ILs is the ability to design these solvents by manipulating the component anion or cation. This is a major reason why ILs are referred to as designer solvents and have been in keen interest in a range of scientific disciplines. This rise in interest has also led to the increased production of ILs, however the toxicity of these designer solvents is not well known. A major concern in utilizing ILs is to develop effective remediation methods in case of environmental contamination. Studies have indicated that potassium permanganate can be used to breakdown pyridinium based derivatives therefore 1-butyl-3-methylpyridinium bromide ([bmpy]Br) was studied and a separation technique was determined. In this research the separation of [bmpy]Br and two reaction intermediates was developed using 5 mM KH2PO4. Further research in the degradation of BUMP can potentially exhibit an extraction method for environmental remediation.

Keywords: Environmental, Environmental Analysis, HPLC, Liquid Chromatography
Application Code: Environmental
Methodology Code: Liquid Chromatography
Breast cancer is a prevalent disease found mainly in women. When the cancer reaches metastasis, it is expensive and difficult to treat. Diagnosis of breast cancer is traditionally done by mammography and prognosis has recently headed to the direction of detecting certain biomarkers in blood or serum, often by RT-PCR. Studies have shown that prolactin-inducible protein (PIP), human epidermal growth factor receptor 2 (HER2) and cytokeratin-19 (CK19) are three among the biomarkers prognostic for breast cancer. Therefore, quantification of these markers could provide important information about breast cancer metastasis. Previous research in our lab has developed a method to detect these biomarkers in buffer and serum samples using molecular beacons (MB). The limit of detection of our assay is improved by preconcentrating the mRNA biomarkers with designed immobilization oligonucleotides. Design of the immobilization oligonucleotides impacts effectiveness of the preconcentration, apparently in part due to overlap of the MB and immobilization oligonucleotide binding regions on the mRNA biomarker as well as the temperature of the elution.
Identifying controlled substances has proven to be a costly and time consuming task for crime laboratories. At the end of 2005, there were 222,678 backlogged requests related to controlled substance analysis alone (Durose, 2008). Additionally, the variation in content of the samples increases the difficulty of analysis. Portable Raman Spectroscopy is an ideal technique for qualitative identification of these samples due to its timely, cost-effective, non-destructive nature. Samples can be analyzed through a variety of containers, minimizing transfer of the sample and preserving the forensic evidence. Furthermore, the ease of instrument operation minimizes the costs of training incurred by crime laboratories, allowing detectives and other officers to complete preliminary identifications in the field, minimizing flow of samples through crime laboratories. The goal of this project is to determine the accuracy, reliability, and precision of portable Raman Spectroscopy in the forensic identification of controlled substances, in particular, “real-world” samples. Utilizing B&W Tek’s Tactic ID®, there was a collaboration with crime labs in the St. Louis, MO community to test these principles, where obtained spectra from samples of controlled substances and mixtures. Comparisons will be made between Raman identification results to those determined by validated analytical methods in the crime lab.


Keywords: Forensics, Molecular Spectroscopy, Raman
Application Code: Homeland Security/Forensics
Methodology Code: Molecular Spectroscopy
The determination of the metal content in paper can be used in forensic identification to trace the paper to suspects. Examples include forged checks, money and last will and testaments. Only a small sample of the document in question is needed to determine the metal concentration, if a database is established, the concentration found can be compared to those in the database to determine the type of paper. By digesting the sample of paper it can then be analyzed by either ICP-OES or ICP-MS, to determine the concentration of specific metals by comparing the values obtained to the standards used. Previous work (1) has been done using ICP-MS as the analytical method, where the limit of detection for the elements was found to be ranging from 1.0 ug/g to 0.001 ug/g. This article showed results for eight elements Mn, Sr, Al, Mg, Ba, Fe, Zn, and Pb. ICP-OES was chosen for this research because it is more cost effective and it is hypothesized that similar results can be obtained. The same elements are analyzed along with Sn, Zr, Ag, Ce, Sc, Th, and Y.

Undergraduate Students Only Poster Session

Simultaneous Detection of Extracellular and Intercellular Potassium Ion Concentration in Whole Blood Using Pulsed Chronopotentiometry

The basis of this research is to develop an effective method to measure potassium ion concentration in plasma and red blood cells (RBC's) simultaneously. Potassium concentration is vital in human health due to its role in biological systems including maintaining osmotic balance between cells and interstitial fluid. A concentration outside of the normal range can lead to muscle weakness, decreased reflex response, and even respiratory paralysis and cardiac arrhythmia. Current studies have shown the importance of measuring both extracellular and intracellular potassium concentrations simultaneously, particularly, for early detection of the development and risk of hypertension.1 The most common methods for potassium measurement used today are classical potentiometry2 and atomic photometry. These techniques are effective to measure potassium in plasma and/or RBC's after sample preparations, but have limitations for simultaneous measurement.

We report here pulsed chronopotentiometry as a fast and reliable method for the measurement of intracellular and extracellular potassium concentrations simultaneously. Here, current pulses of varying magnitude are applied to extract ions from the sample into the membrane. Depletion of ions at the membrane surface occurs when the concentration of ions in the solution can no longer sustain the flux of potassium across the membrane and this point is detected by a potential drop on the potential-current response curve.3 This limiting-current is proportional to the potassium ion concentration according to Sand equation. After measuring potassium concentration in unlysed blood, the same sample is lysed and re-measured, giving the total concentration. Simple arithmetic allows for intracellular concentration to be calculated.


Keywords: Bioanalytical, Detection, Electrochemistry, Ion Selective Electrodes
Application Code: Bioanalytical
Methodology Code: Electrochemistry
In this study, we employ the principles of ion transfer across liquid/liquid interfaces to observe ion-selective currents of thyroid hormones with micro and nanopipette electrodes. The selective detection of thyroid hormone, thyroxine, in sodium phosphate buffer solution and serum is demonstrated via cyclic voltammetry through ion transfer at the water/1,2-dichloroethane interface. Our motivation originates from the importance of developing rapid analysis systems for hormone levels in the blood and will expand applications of point-of-care testing. Electrochemical measurements with micro and nanopipette electrodes are especially suited to point-of-care testing due to rapid analysis times, simple calibration, and ability to provide hormone concentration levels in blood serum. Thyroxine hormone is of particular interest due to its clinical relevance as a marker for thyroid disease. Untreated thyroid disease can have disastrous effects on an individual's health and lead to other autoimmune diseases such as type II diabetes. Future work in the development of electrodes selective for triiodothyronine and thyroid-stimulating hormone will also be presented.
Bourbon whiskey is made from grains such as corn, rye, wheat and malted barley. After fermentation, the resulting white whiskey, or “white dog”, is matured in new charred-oak casks. During the process of maturation, the whiskey picks up its distinct flavor and coloring from the oxidized oak. It is the variety and differing composition of these organic flavor components that distinguish each brand of whiskey. Various spectroscopic methods have been devised in order to monitor quality of flavor and alcohol content of whiskey due to the fast and non-destructive nature of the technique. Numerous techniques have relied upon absorption in the near-infrared to the visible to identify a variety of analytes in alcoholic beverages. Fluorescence spectroscopy offers the potential for an additional means for a fast, efficient, and non-destructive way to quantitate a variety of complex fluorescent species present in the whiskey sample. Specifically, synchronous scan fluorescence spectroscopy is a technique that can result in the formation of narrow spectral bands and has the capacity to handle the simultaneous analysis of the numerous fluorophores present in the whiskey sample. The work presented herein reveals the influence of solvent polarity on the resolution of the SSF emission of the various organic fluorophores in whiskey, and also evaluates the solvatochromic shift that occurs in the presence of a variety of solvents.

Keywords: Analysis, Consumer Products, Fluorescence, Spectroscopy
Application Code: Consumer Products
Methodology Code: Fluorescence/Luminescence
Gabapentin and bexarotene are both under investigation as treatments after spinal cord injury. Gabapentin is being studied for use after high-level spinal cord injury (above thoracic level six) to prevent abnormal neuron sprouting and aberrant synaptogenesis which causes a life threatening condition Autonomic Dysreflexia (AD). Without treatment, AD causes high blood pressure which can induce stroke, seizures, and even death. Bexarotene is being studied as a therapy to concurrently activate signaling pathways that suppress destructive inflammation while promoting activation of tissue repair functions in macrophages. It is expected that activation of these signaling pathways will attenuate secondary tissue damage at/nearby the site of injury and will enhance axon growth and plasticity culminating in improved recovery of neurological function after spinal cord injury. High performance liquid chromatography electrospray ionization time-of-flight mass spectroscopy (HPLC-ESI-TOF-MS) was used for the quantification of gabapentin or bexarotene in serum samples. Solutions of samples were prepared and concentrations of drug were determined on the basis of calibration curves created using standards prepared in tissue. The samples were spiked with an internal standard directly after tissue harvest and prior to any further manipulation to correct for variations in sample preparation and analysis. This separation and detection method is suitable for quantitative sample analysis at low concentrations. With the developed method, it has been shown that HPLC-ESI-TOF-MS is capable of determining the concentrations of gabapentin or bexarotene in serums, and this method of detection will assist neuroscientists in the determination of efficacious dosages as well as administration methods.

Keywords: Biological Samples, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
The nucleobase guanine has proven to be extremely unique due to its ability to form complex secondary structures known as G-quadruplexes. G-quadruplex structures are comprised of stacked guanine tetrads, which form via the association of four guanines through Hoogsteen hydrogen bonding. DNA sequences rich in guanine are capable of forming such structures, and a number have been confirmed in vitro. Recent evidence has strongly supported G-quadruplex formation in vivo as well. Many of the G-quadruplex structures confirmed in vitro have been found to function as aptamers, oligonucleotides that bind target molecules with high affinity and specificity. Furthermore, guanine-rich regions are prevalent within the human genome, particularly within the promoters of human oncogenes, and have been determined to have regulatory significance. Therefore, these regions represent areas of interest and may provide a source of potential aptamer sequences. In this research we aim to identify the conditions that govern the stability, formation, and aptamer capabilities of G-rich human oncogene promoter regions. Conditions of interest include pH, temperature, and small molecule interactions. Formation of G-quadruplex structures is assessed by circular dichroism (CD) spectroscopy. Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is used to screen for protein capture by the G-quadruplex-forming DNA sequences. By better understanding the effects of these conditions, we aim to improve the use of G-quadruplex structures as aptamers for the diagnosis of diseases associated with their target proteins, as well as to develop tools to analyze and study the biological significance of G-quadruplex structures within oncogene promoter regions.

Keywords: Nucleic Acids, Protein, Spectroscopy, Time of Flight MS
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Undergraduate Students Only Poster Session

Affinity Capture of Proteins at Aptamer-Modified Surfaces Using a MALDI-MS Platform

Aptamers are short, single-stranded nucleic acids that specifically bind to target molecules with high affinity. They derive their functionality from the unique secondary structures they form, such as the G-quadruplex structure. In this research project, the processes that govern the affinity capture of proteins at aptamer-modified surfaces are studied using an affinity Matrix-Assisted Laser/Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) platform developed in our lab. In our method, the aptamer sequence is covalently attached to a fused silica or indium tin oxide (ITO)-coated surface. After incubating the surface with target proteins or protein mixtures and rinsing off any unbound or weakly bound species, MALDI matrix is added. Captured proteins are then analyzed directly at the aptamer-modified surfaces by MALDI-TOF MS. Our initial studies focus on the thrombin binding aptamer (TBA), which binds to thrombin via G-quadruplex formation and functions as a model for aptamer behavior. Various parameters, including aptamer surface coverage, target protein concentration, linker concentration, incubation time, and rinsing procedure, have been investigated and were found to affect the binding processes of the TBA-thrombin system. By determining the optimal conditions for protein capture by aptamers, we aim to develop a better understanding of the intricacies of these affinity interactions. These optimized conditions can then be applied to the investigation of other aptamer systems, as well as potential aptamer sequences based upon the G-quadruplex structure, such as the c-myb and c-myc oncogene promoter region sequences. Such insight into the inner workings of aptamers will lead to the development of more sensitive biosensor devices and more accurate diagnosis of diseases associated with these captured proteins.

Keywords: Mass Spectrometry, Nucleic Acids, Protein, Time of Flight MS
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
The acquisition of metals is essential for a variety of protein and cellular functions, including sensing of environmental conditions. The single-celled green algae Closterium moniliferum selectively accumulates the toxic, heavy metals barium and strontium, which are then incorporated as barium sulfate crystals as statoliths in the terminal vacuoles. Thus, C. moniliferum may be used to contain accidental release of Ba and Sr into the environment from industrial activities, such as hydraulic fracturing. Hydraulic fracturing technology collects natural gas through the injection of hydraulic fracturing fluid into shale rock. When the flowback water and produced water are collected, they are enriched in dissolved Ba and Sr. These metals are concentrated to thousands of milligrams per liter. To assess its candidacy for remediation, this study sought to characterize the C. moniliferum’s growth, intracellular uptake rates of Ba and Sr, and absorption of Ba and Sr onto the cell wall. The algal population growth was negatively correlated with the percentage of fracking fluid present in the media. However, the population was sustained in as much as 1% hydraulic fracturing fluid. Algae cells were cultured in a variety of media, including media enriched in Ba, Sr, or frack water. Cytosolic Ba and Sr accumulation was determined using atomic spectroscopy. Additionally, Ba and Sr surface absorption to the exoplasmic cell wall was investigated. While known to be incorporated as vacuolar statoliths, Ba’s and Sr’s interactions with surface sugars and proteins are poorly understood. Fourier transformed infrared spectroscopy (FTIR) was utilized to characterize functional group stretching in response to Ba and Sr binding. Hydroxyl groups were primarily responsible for Ba and Sr absorption. The results suggest C. moniliferum should be continued to be investigated as a means of passively remediating hydraulic fracturing contamination.

**Keywords:** Atomic Spectroscopy, Environmental/Biological Samples, Environmental/Water, FTIR

**Application Code:** Environmental

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
Abstract Text
The primary objective of this research was to develop a method of obtaining accurate spectra from a liquid crystal tunable filter (LCTF) microfluorometer. Like with any instrument, the various components of this instrument displayed non-ideal behavior. By comparing the raw spectra from the instrument to one from a conventional instrument, a set of proportionality constants was generated that served to correct all future spectra taken by the microfluorometer. By overcoming artifacts in the light source, filter, and camera, the spectra from this microfluorometer were found to be in agreement with spectra obtained by conventional instruments. This research utilizes this instrument’s ability to study the fluorescence of micro-volumes in order to determine varying fluorescence between microenvironments. Fluorophores that are added as a stain to cell membranes can be distinguished from the same fluorophore in solution around the cell, due to the effects of each of these two environments. Current efforts are aimed at the generation of a calibration curve to measure the concentration in micro-volumes of fluorescent species.

Keywords: Fluorescence, Liquid Crystal, Microscopy, Standards
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
In this study including the field of fingerprinting metabolomics approach for food safety and quality assessment, the evaluation of untargeted compounds using LC-ESI/TOF/MS and multivariate statistical analysis method is proposed for the assessment of classification, contamination and degradation of infant formula. LC-MS fingerprinting metabolomics is the method judging from the statistic of the principal components analysis (PCA) of the mass charge detected by mass spectrometric detection of the separated compounds by chromatographic technique. This study shows that this methodology can be applied for the food defense regarding to the contamination of unknown contaminations and degradations of infant formula samples. A few reports were discussed that LC-MS metabolomics was used for the classification of origin, process and other effects for various agricultures. Thus, in this study, the fingerprinting metabolomics is applied to evaluate the safety and quality assessment of infant formula. For the detection of many peaks using LC-MS, the reversed phase and HILIC modes are used to monitor more detected numbers in infant formulas in the positive scan mode than the single phase. The repeatability of the non-targeted contents from 4 kinds of infant formulas based on the principal components analysis (PCA) was less than the relative standard deviation of 15% for all groups. The PCA pattern showed that significant differences in the classification of types and origins, the contamination of melamine, pesticides and several metals, and the degradations for one week and expiration date were evaluated using fingerprinting LC-MS metabolomics. With this strategy, the differences from the untargeted compounds could be utilized for quality assessment of infant formula.

**Keywords:** Food Safety, Liquid Chromatography/Mass Spectroscopy  
**Application Code:** Food Safety  
**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Characterization of the Stability of Supported Bilayer Membranes in Polydimethysiloxane Microfluidic Devices

Supported bilayer membranes (SBMs) have been used to coat capillaries and microfluidic devices. These coatings are similar to cell membranes and prevent adsorption of proteins, DNA, and other biomolecules to channel walls. Combined with the small dimensions of microfluidic devices, such coatings are an optimal tool for biological and biomedical research. To characterize the stability of SBMs, we prepared hybrid PDMS-glass devices containing straight, 3 cm channels. The channels were filled with small unilamellar vesicles, which self-assemble into supported bilayers. Conductivity measurements of the electroosmotic flow were used to characterize the presence and stability of the SBMs. Our primary focus was the stability of coatings using natural vs. synthetic lipids. Preliminary results indicate that the synthetic lipid coatings are more stable than the natural lipid coatings, suggesting that the variations in length and saturation of natural lipid tails may affect the stability of the coatings. Additional studies are in progress to confirm these results. Electroosmosis measurements of the two lipid coatings were relatively stable (RSD 8-18%) for 2-4 hours when a constant voltage was applied across the channel. At longer time points, electroosmotic mobility increased to that of a bare chip suggesting the coating was destroyed. The stability of these membranes was also studied over a two week period to determine how long a device can be stored before use. Our results suggested that a chip can be stored for about a week and still yield consistent results. After a week, the coatings deteriorated, as evidenced by increasing electroosmotic mobility. On-going work is exploring effects of cholesterol on SLBs to further enhance the stability and storability of these coatings.

Keywords: Electrophoresis, Lab-on-a-Chip/Microfluidics, Lipids, Membrane
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Across the world synthetic drugs are becoming increasingly popular. As forensic research and crime labs alike identify questionable substances, there is a steady production of newer products being synthesized to fill the niche of ‘legal highs’ desired on the black market. These drugs are appealing to people who are looking for ways to pass drug screenings and avoid legal issues; all while ignoring rather severe health risks. If a greater number of unknown substances can be identified, the sooner such compounds can be scheduled and banned. Through liquid and gas chromatography/mass spectrometry, nuclear magnetic resonance spectroscopy, infrared spectroscopy, and Electrospray ionization-MS/MS/MS studies, a number of synthetic cannabinoid and synthetic psychoactive compounds will be studied. The test matter will be obtained from shops within Western Pennsylvania and West Virginia. The research will be performed under Profession Michael Cipoletti, Program Director of Forensic Science at Waynesburg University. Most synthetic compounds, taken as drugs, are very dangerous. This is most often overlooked by users, due to the so-called legality of such substances. Similar discovery and identification research needs to continue to grow in order to lead to the banning of all synthetic drugs. Studies that elucidate and characterize unknown synthetic drugs can help lead to the discovery of producers and distribution sites throughout the world.

Keywords: Drug Discovery, Electrospray, Forensics, Mass Spectrometry
Application Code: Drug Discovery
Methodology Code: Mass Spectrometry
Glassy carbon (GC) electrodes were modified with substituted aryl groups via a one-step synthesis and grafting procedure developed in our lab. This procedure involves grinding the electrode in a water paste of sodium nitrite, p-toluenesulfonic acid and an aryl amine, such as p-nitroaniline, m-nitroaniline, 2-aminoanthraquinone, 4-aminosalicylic acid or 4-aminobenzoic acid.

Voltammetry of modified electrodes showed peaks for attached nitrobenzene and anthraquinone groups. Raman spectra of the resulting paste included a peak near 2300 cm$^{-1}$, indicative of a diazonium group. Addition of 2-naphthol to the paste gave intensely colored products, indicative of azo dye formation. These results suggest diazonium ion formation followed by electrode grafting perhaps by either a radical or cationic mechanism.

After modification, electrodes were cleaned ultrasonically; dilute acid was found insufficient for removing weakly bound groups, while 10 minutes of sonication in acetone was effective. After sufficient cleaning, the coverage of p-nitrophenyl groups on GC was about 0.5 nmol/cm$^2$ (near monolayer) as determined by integration of voltammetric peaks.

Ferrocenecarboxylic acid and dopamine electrochemical reactivity was not significantly hindered at the modified electrodes. Surfaces modified with p-carboxybenzene groups were tested for their ability to preconcentrate copper ions. Enhanced binding of copper on grafted GC compared to bare GC was observed; however the copper response was considerably lower than that seen on electrografted surfaces, likely due to lower surface coverage and thus fewer binding sites available to the metal ions.

This work was made possible by the Virginia Ellis Franta Fund for Chemistry.
Behavior of Calcium and Magnesium Ions in the Great Miami River, Ohio

The Great Miami River is dominated by agricultural land use with lime applications in the late winter and spring months. The objective of this study was to examine seasonal flow, and the behavior of calcium (Ca\(^{2+}\)) and magnesium (Mg\(^{2+}\)) in the Great Miami River at Hamilton, Ohio (9,402 km\(^2\)). Using an automatic sampler, 280 water samples were collected between January 2013 and May 2014 by the Miami Conservancy District. Samples were filtered (0.4 um) and analyzed for major cation concentrations using an Ion Chromatograph at Wittenberg University. Median Ca\(^{2+}\)+Mg\(^{2+}\) concentrations were lowest in the spring (0.8 mM), associated with the highest median flow during sample collection (89 m\(^3\)/s). Median seasonal Ca\(^{2+}\)+Mg\(^{2+}\) yields were highest during the winter (34,000 kg/h) and similar during the spring, summer, and fall (22,400 kg/h; 20,200 kg/h; 21,100 kg/h). The molar ratio of Ca\(^{2+}\)+Mg\(^{2+}\):Mg\(^{2+}\) was examined versus flow for each season to identify enrichment associated with calcite (CaCO\(_3\)) lime applications. Spring calcium-enrichments were as much as double winter values at similar flow. Interestingly, the median enrichment for summer falling limb samples (3.5) was higher than the median for the rising limb (3.0). Chemical weathering of agricultural lime in the spring and recharge of summer groundwater likely explain enrichment trends. As with other agricultural watersheds in the Mississippi River Basin, the area-normalized yield of Ca\(^{2+}\)+Mg\(^{2+}\) was elevated with respect to yields in forested watersheds. This is similar to trends observed in alkalinity, and consistent with lime enrichment.

Thank you to the Virginia Franta Fund for supporting this research effort.

Keywords: Agricultural, Environmental, Environmental/Water, Ion Chromatography
App. Code: Environmental
Method. Code: Liquid Chromatography
Analytical techniques for the purification have been highlighted in the biomedical and food engineering. In the early 1970s, counter-current chromatography (CCC) was reported in pioneering work by Dr. Ito. This analytical technique provides an advantage over the chromatographic purification by eliminating the use of a solid stationary phase that is limited and dangers of irreversible adsorption. In 1980s, the CCC technique has been improved in terms of resolution, separation time and sample loading capacity, then these is named to high-speed CCC (HSCCC) Recently, HSCCC is accepted as an efficient preparative technique, and widely used for the purification of various natural and biological products. In this study, we apply this techniques for the purification of natural and food components such as crocetin and malachite green. Thus, this technique has been useful for the purification of all-purposed chemical components from various products. In this application, specific information and basic data of HSCCC would be discussed.
Undergraduate Students Only Poster Session

Measurement of Neuronal Hydrogen Peroxide Release in Chemotherapy Treated Rats

Post-Chemotherapy Cognitive Impairment (PCCI) occurs after pharmacological chemoagents are administered to fight carcinomas. PCCI entails a general decline in complex problem solving, learning, memory, and motor function in up to 30% of patients who receive chemotherapy treatment. Previous studies in our group have found dopaminergic neurons are unable to release proper amounts of the neurotransmitter dopamine upon stimulus after treatment with certain chemoagents. Furthermore, the dopamine transporter experienced a similar attenuation. Here, oxidative free radical activity was investigated by probing for spontaneous hydrogen peroxide release in chemotherapy treated rats using fast-scan cyclic voltammetry. Neurochemical analyses were performed after rats underwent chemotherapy treatment with 20 mg/kg injections of Carboplatin for four weeks. Mercaptosuccinic acid, a glutathione peroxidase inhibitor, was applied to brain slices of saline and chemotherapy treated rats to induce an increase in oxidative activity. Spontaneous hydrogen peroxide release appears to increase in chemotherapy rats, thus indicating a potential source of the neurocognitive symptoms of PCCI.

Keywords: Bioanalytical, Electrochemistry, Voltammetry
Application Code: Biomedical
Methodology Code: Electrochemistry
Pharmaceuticals and their degradation products have been observed in the environment, where they pose potentially negative effects to humans and other organisms. Degradation products may be more environmentally persistent and more toxic than their parent compounds. To investigate the environmental fate of a commonly prescribed antidepressant, we measured the solar photodegradation rate of sertraline (Zoloft®) (SER) in samples simulating the natural aquatic environment. Aqueous drug samples were prepared in 5 mM phosphate buffer (pH=7.0), with and without the organic degradation product humic acid (HA). These samples were photoexposed in a solar simulator and aliquots were taken at relevant time points followed by analyzed using HPLC and LC-ESI-MS. The photodegradation of sertraline followed a pseudo-first order rate law, as expected; using this data, the half-life of sertraline in the various aqueous solutions was calculated. We observed that HA acted as a photosensitizer, speeding up the photodegradation of sertraline. Products of SER photodegradation were detected and structures proposed.
The use of enzymes in analytical assays is both expensive and inconvenient requiring extraction, purification and storage in specific conditions of temperature and pH. Nanomaterials-based enzyme mimics have attracted considerable interest as alternative catalysts to natural enzymes. This presentation will report studies of the oxidase like properties of nanoceria particles and their use as analytical probes for the detection of phenolic compounds. We will describe a comparative analysis of the oxidase like activity in relation to the physicochemical characteristics of these particles, and define conditions of use in terms of reaction conditions, buffer composition and pH, that is essential for the future use of these particles in analytical assays. Applications of this method for the detection of dopamine and catechol selected as model phenolics will be demonstrated. Performance of this method will be compared to that of an enzyme based detection of the two compounds. The results demonstrate that nanoceria particles can be used to replace oxidase enzymes in the development of cost effective and sensitive methods for the detection of these compounds.
Pharmaceuticals enter wastewater treatment plants as the result of human excretions or improper disposal and are often not wholly removed during the treatment process. These compounds then enter the aquatic environment and are exposed to sunlight, which facilitates photochemical degradation. In this work, the photodegradation of diphenhydramine and ranitidine, two over-the-counter medications, was investigated in dilute aqueous solutions. Solutions were buffered at environmentally relevant pH levels; humic acid was added at varying concentrations in order to simulate natural water samples. All solutions were exposed to light in a solar simulator with aliquots taken at preset time points and analyzed by both HPLC and LC-ESI-MS. The rates of photodegradation were found to follow pseudo-first order rate laws and the half-life of each compound under different conditions was determined. LC-ESI-MS and MS/MS were used to detect and identify the products of photodegradation.
Chemometrics is the application of multivariate analysis on complex chemical data sets. Spectroscopic instruments have the ability to produce large amounts of information on analytes. However, it is common to only utilize one wavelength for analysis, and this approach cuts out a large portion of the data. Chemometrics offers a way to analyze samples while using all the information contained within the spectra. Chemometrics is generally not taught to undergraduates and is often approached in industry and research settings as a black-box. The black-box approach refers to analysts using chemometrics without understanding the algorithms and assumptions being applied as part of the model. This work has been an undergraduate interdisciplinary research project between chemists and statisticians spanning two years. Understanding the algorithm of principle component analysis (PCA), learning how to collect samples for chemometric methods, and applying PCA for chemical predictions have been goals of this project. The research being presented is the use of PCA to study fluorescence of glucose oxidase complexed with 8-anilino naphthalenesulfonic acid and laser induced breakdown spectroscopy of barium and strontium in frack water precipitates.

Keywords: Chemometrics, Fluorescence, Spectroscopy, Statistical Data Analysis
Application Code: General Interest
Methodology Code: Chemometrics
# Abstract

While ozone in the stratosphere is essential for life on earth by blocking harmful ultraviolet radiation, tropospheric ozone has many negative effects. Ground level ozone contributes largely to smog formation and is responsible for 90% of annual pollution-related crop loss. It has many acute and chronic biological effects on plants, including inhibition of photosynthesis. A multi-week high school chemistry lab was designed to demonstrate the effects of electrochemically produced ozone on golden pothos (Epipremnum aureum), a common indoor house plant. Photosynthesis and respiration rates were analyzed by Vernier CO\(_2\) and O\(_2\) gas sensors both before and after exposure of the plant to ozone. Atmospheric ozone concentrations were also determined using colorimetric analysis with Schoenbein paper. This multi-week experience will not only demonstrate comprehension of basic chemistry concepts presented in the course but also real-world environmental issues in today’s society.

**Keywords:** Education, Electrochemistry, Environmental/Air, Method Development

**Application Code:** Environmental

**Methodology Code:** Education/Teaching

**Session Title:** Undergraduate Students Only Poster Session

**Abstract Title:** Developing a High School Lab Experience to Analyze Environmental Effects of Ozone

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**Date:** Monday, March 09, 2015 - Afternoon

**Time:**

**Room:** Exposition Floor, Hall F, Aisles 390
The ongoing use of cell phones and other electronic devices leads the scientific community to wonder how these technologies affect the users. While in vitro studies are limited in describing how an organism is directly affected by the microwave radiation produced by these devices, they are helping in determining how biomolecules are altered in response to the exposure. One biomolecule, glucose oxidase, (GOX), is used for medical purposes in glucose meters; thus its stability is key for its accuracy. Microwave exposure of GOX and the resulting structural changes can be studied through fluorescence spectroscopy, involving an ANS probe and tryptophan residue analyses. The GOX aqueous samples are exposed to 150 W of microwave radiation for 15 minutes before fluorescence analyses. Preliminary studies have demonstrated that the fluorescence intensity changes significantly, for both analyses, upon microwave exposure relative to the native GOX state and relative to the thermal control. Further work involves fluorescence analysis during exposure as well as renaturation studies of GOX.

Keywords: Analysis, Fluorescence, Microwave, Protein
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
The choice of basis set is one of the most important choices made in a computational calculation because of its effects on the way each quantum mechanical calculation is executed. Several types of basis sets exist, each defining the system with a distinctive mathematical approach for the energy calculation. The choice of basis set typically depends on the type of model and the level of accuracy needed or wanted. In this work, we show the variance in basis set choice on the accuracy of vibrational frequencies (VF) in several small molecules. We use the computational software package, Gaussian 03 using WebMO 14, running Density Functional theory, and two Coupled Cluster theories, (CCSD and CCSD(T)). The basis sets assessed are, cc-pVDZ, and cc-pVTZ which were chosen because of their inherent accuracy in the calculated values. Data were then compared to standards from the National Institute of Standards and Technology (NIST) experimental VF database. Our results suggest that the most appropriate basis set choice is cc-pCVTZ with an average absolute error of 1.94% relative to NIST experimental data. We also examined the efficiency of our Gaussian 03 program, which is determined to have an average absolute error of 0.648% compared to the NIST computational database standards. We conclude that for small molecule VF, the most appropriate and accurate basis set is cc-pCVTZ, and that results from our modeling program are statistically similar to NIST values. Funding was provided by the Westminster College Chemistry and the Westminster College Drinko Center for Experiential Learning.
# Evaluation of Truncated Peptides for the Enhancement of Fluorescence Polarization Immunoassays

Immunoassays are a class of ligand binding assays that quantify the presence of an antigen of interest through an interaction with a specific antibody (Ab). Immunoassays can be performed in several formats; a popular implementation is the use of fluorescence polarization (FPIA) detection. In an FPIA, fluorescently labeled antigen is used to report on binding events. Upon excitation with a controlled polarization of light, the degree of retained polarization upon fluorescence emission will depend on the degree of rotation of the molecule in solution. In respect to the immunoassay mixture, the labeled antigen will rotate fast when not bound, producing a high degree of depolarization. However, when bound to a large Ab, the rotation will be retarded, and the depolarization will diminish. For a sensitive FPIA, a large change in size upon binding is necessary to elicit a large change in polarization.

In this work, we tested if truncation of a fluorescently labeled insulin could optimize an insulin FPIA. The Ab-binding domain, located at the C-terminus of the beta chain, was retained while the alpha chain was removed. With this modification, a smaller peptide resulted that exhibited a larger depolarization of light in the absence of Ab. In the presence of Ab, the polarization increased as expected. To characterize binding of Ab to the modified peptide, we titrated a solution of modified peptide with Ab while monitoring the polarization. A Scatchard analysis revealed a $K_{d}$ of 500 nM. The binding affinity following the modification depicted lower affinity than the full insulin molecule ($K_{d} = 8$ nM), yet it is promising as a complementary tool for FPIA.

## Keywords
- Fluorescence
- Immunoassay
- Peptides
- Spectroscopy

## Application Code
- Bioanalytical

## Methodology Code
- Fluorescence/Luminescence
Polydimethylsiloxane (PDMS) microfluidic devices have been developed as diagnostically useful, disposable, and portable “Lab on a Chip” (LoC) devices. LoC devices are fabricated for a wide variety of functions including immunoassays, analysis of bodily fluids, and DNA amplification. The current fabrication method for PDMS chips utilizes photolithography with a transparent photomask containing the desired device design, which creates a negative mold for the PDMS. Currently, when a design needs to be redesigned, a new photomask must be created. Photomasks are commonly made by outside facilities and can delay progress by days or weeks. A modular design of these photomasks could eliminate this delay by providing many possible arrangements and options of the design during fabrication.

The modular method consists of utilizing multiple masks containing simple elements, which when combined, produces a final design that achieves the desired channel structure. The various masks join at specific points when the photomasks are aligned, allowing for the various combinations. We demonstrated this methodology by successfully fabricating a device that has two inlets and one mixing channel to produce aqueous droplets in an oil carrier phase. These droplets have been shown to be powerful high-throughput microreactor systems that reduce reagent consumption, have the capability of producing multiple reactions, and decrease reaction times. The modular nature of this method allows rapid modification of photomasks for a greatly reduced device-to-device turnaround time.

Keywords: Lab-on-a-Chip/Microfluidics
Application Code: Other
Methodology Code: Microfluidics/Lab-on-a-Chip
A Group of Uniform Materials Based on Organic Salts (GUMBOS) encompasses organic solid–state salts which exhibit properties similar to those of ionic liquids (ILs). The tunable characteristics of GUMBOS permit applications in chemical, technological, and biological disciplines. As previously reported by our group, carbazole-derived GUMBOS are good hole transport and emissive materials for applications in blue organic light emitting diodes (OLEDs). The unique luminescent and electrochemical properties of carbazole-based GUMBOS enable potential use of these materials in OLEDs. Herein, syntheses of nanoparticles derived from carbazole-based GUMBOS (i.e., nanoGUMBOS) using templated and non templated ultrasonication approaches are described. The carbazole-based cation was coupled with different counteranions: iodide ([I]), trifluoromethanesulfonate ([OTf]), and (pentafluoroethylsulfonylimide ([Beti]). The average sizes of these nanoGUMBOS were determined using transmission electron microscopy and the optical properties were evaluated by use of absorbance and fluorescence spectroscopy. Absolute photoluminescence quantum yields were also measured using an integrating sphere. The size range of nanoGUMBOS was from 49 nm up to 204 nm depending on the synthetic method and experimental conditions. The molar extinction coefficient and fluorescence intensity, as well as quantum yields, were tuned with the synthetic conditions and average sizes of nanoGUMBOS. The highest quantum yields were reported for [Cl][Beti] (14 %) as compared to [Cl][I] and [Cl][OTf]. These size-dependent optical properties have allowed us to widen the potential applications of these organic nanomaterials for use in optoelectronic devices such as OLEDs.

The authors acknowledge NSF for funding under grant CHE-1307611.
Nitroanilines are used as chemical intermediates in the manufacturing of dyes, pesticides, gasoline, specific pharmaceuticals, and poultry medications. There are three isomeric forms of nitroanilines that differ from one another in the position of the nitro substituent. The three isomers, namely 2-nitroaniline, 3-nitroaniline, and 4-nitroaniline, can be distinguished by Raman spectroscopy. The Raman signal can be enhanced by the use of gold or silver nanoparticles in a technique known as Surface Enhanced Raman Spectroscopy (SERS). In this project, colloidal gold nanostars and Klarite™ substrates based on nanoscale patterning of a gold coated silicon surface were used for SERS analysis and characterization of the three isomers. Even though the three nitroaniline isomers have similar structures, their SERS spectra were easily distinguishable from one another and from aniline. The degree of Raman signal enhancement were of the order of 2-nitroaniline > 3-nitroaniline > 4-nitroaniline. The enhancement effects of colloidal gold nanostars versus Klarite™ substrate was compared. The enhancement factors of the different isomers were used to investigate the steric hindrance effects associated with nitroaniline chemisorption as well as the inductive effects due to the position of the nitro substituent group. Computational modeling based on Density Functional Theory (DFT) was also conducted to study the adsorption characteristics of the analytes on gold colloid.
Phosphorus is an essential nutrient for maintaining a healthy ecosystem; however, heightened levels of phosphorus can have negative effects, including algal blooms, which upon decomposition, can lead to oxygen depletion, resulting in regions lacking aquatic life. It is therefore important to monitor phosphorus levels in bodies of water. Current quantitative methods for phosphorus detection in water are based on the standard method 4500-PE, which detects phosphorus colorimetrically through the reduction of an antimony-phosphomolybdate complex. This standard method, however, often requires sample preservation along with a large amount of reagents. In addition, an absorbance spectrophotometer must be employed for sensitive detection. In order to develop a more rapid, low-cost, on-site device for detection, a paper-based fluidic device (PFD) has been developed requiring microliter reagent volumes for detection of phosphorus as phosphate using a modified ascorbic acid reduction method. These PFDs are smaller than a business card, and are fabricated on cellulose filter paper using a wax printer and a hotplate to develop channels for reagent mixing. Upon mixing of phosphate-containing sample and a combined reagent consisting of sulfuric acid, ammonium molybdate, potassium antimonyl tartrate, and ascorbic acid, a blue color develops. The intensity of the blue color is directly related to the phosphorus concentration, which was measured visually or through densitometer software. It has been found that as little as 150 ppb phosphorus can be detected. This method can be applied in the field to detect and possibly pinpoint sources of nutrient loading into a body of water.
Abstract Text

The need for a better method of blood drawing and analysis is increasing for the demands of the aging population in the US. By utilizing dried blood spot (DBS) cards and media, a smaller amount of blood is required and can be taken and shipped by the patient for analysis. This allows analytes in blood to be quantified with small amounts of sample. Sampling of blood for DBS analysis is also simple and can be completed by the patient at home by finger or heel pricks. Previously, the DBS cards had to undergo microwave digestion to analyze the inorganic analytes in blood using inductively coupled plasma–mass spectrometer (ICP-MS). However, using an excimer laser, the blood can be ablated from the card and analyzed directly, cutting out hours of the sample preparation work. Accuracy in laser ablation (LA)–ICP-MS can be achieved by utilizing isotope dilution mass spectrometry (IDMS), which eliminates the use of calibration curves and allows the standard to be on or in the matrix. This method has been standardized and is covered in EPA method 6800 update V, 2013. LA can also be used to quantify organic molecules and peptides in whole blood that play important roles in maintaining human body functions by coupling it with a molecular mass spectrometer, such as Q-ToF-MS. The quantification of peptides allows a better understanding for doctors to more often follow their patients and improve treatment. IDMS is used for the quantification of both inorganic and organic ions.

Keywords: Bioanalytical, Laser, Mass Spectrometry, Quantitative
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Thin silicon nitride films are a common building block of nanofabricated devices. They offer favorable physical properties, such as the ability to form free-standing <100nm-thick films, but their complex surface chemistry presents challenges. We developed a process to electrolessly plate thin gold films onto silicon nitride membranes, and into through-membrane pores, so that we could use thiol-based chemistry to more readily modify the surface chemistry. We discovered that the gold film grain structure allowed us to perform surface-enhanced Raman spectroscopy (SERS). Thermal annealing of the films dramatically changed the gold film structure, as revealed by both the SERS signal and field-emission scanning electron microscopy (FESEM). Mean grain radii, for example, could be readily increased by tens of nanometers by post-deposition annealing under suitable conditions. We exerted control over the film spatial distribution across longer length scales by preceding the plating with a photochemical surface protection route that allowed us to generate patterned gold films. We will describe the various gold film plating approaches and present applications.

This research has been supported by NSF CAREER award CBET-1150085, in part by NSF EPSCoR Cooperative Agreement #IIA-1330406, and by the University of Rhode Island.
This project focuses on the inhibitory effects of dieckol, a phlorotannin found in [i] E. cava [/i], on [alpha]-glucosidase. The use of [alpha]-glucosidase inhibiting drugs like acarbose, miglitol, and voglibose have been shown to reduce the activity of the protein by competitive or non-competitive inhibition. Through cloning and mutagenesis of the [alpha]-glucosidase gene, it is possible to change the structure of the protein and explore the binding site of the dieckol inhibitor. Mutants have been selected to help determine the specific amino acid residues essential for binding dieckol by testing their activity in the presence and absence of the dieckol. The mutants will undergo enzymatic assays with and without dieckol to analyze difference in binding affinity and inhibition between the wild type and mutant enzymes.

Keywords: Biomedical, Enzyme Assays, Ion Exchange, Liquid Chromatography
Application Code: Biomedical
Methodology Code: Other
Multiple cationic biocides used to inhibit microbial growth are commonly incorporated into multipurpose contact lens solutions (MPS). The most widely used cationic additives are polydisperse, complicating their analysis using conventional HPLC with UV detection. This study investigates the use of Ultra Performance Liquid Chromatography (UPLC) coupled with UV detection and electrospray Quadrupole Time-of-Flight Mass Spectrometry (QTOF-MS) for the analysis of the polycationic biocides including polyhexamethylene biguanide (PHMB). The resulting UPLC methods developed were found to provide sensitive and reproducible measurements at sub-ppm concentrations with UV detection when authentic MPS samples were prepared via weak cation-exchange solid phase extraction (> 92% recovery) and analyzed via the method of standard additions (R² > 0.95). Further, mass analysis of the eluting UPLC peaks has provided mass identification of multiple structures from the PHMB polymer, with oligomers ranging in size from 2 – 6 units. Uptake studies of PHMB and alexidine dihydrochloride have also been performed with a range of lens materials. Lenses immersed in stock biocide solutions in conventional contact lens cases for varying amounts (i.e., up to 96 hours) were analyzed by UPLC to ascertain the remaining biocide concentration. The results demonstrate substantial uptake of these biocide materials by the contact lens, resulting in a significantly reduced concentration of biocide in the lens cases. The possibility of preferential distribution of PHMB in particular as a function of oligomer size/length is also explored.

Keywords: Bioanalytical, Liquid Chromatography/Mass Spectroscopy, Time of Flight MS
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
The development of novel transition metal complexes with high binding affinities to DNA aims to resolve many of the therapeutic issues faced by traditional metal-based drugs (e.g., cisplatin) known to suffer from poor selectivity and elevated toxicity. Here we describe results of DNA binding and replication inhibition for TM complexes, including [Cr(diimine)][sup]3+[/sup] systems that display long-lived [sup]2[/sup]Eg excited state lifetimes and strong excited-state oxidizing power. Owing to their unique properties, UV excitation of these Cr(III) complexes in the presence of DNA results in emission quenching and leads to strand cleavage, and can further result in adduct formation. In investigating the disposition of TM complexes toward DNA, Capillary Gel Electrophoresis (CGE) and Polyacrylamide Gel Electrophoresis (PAGE) have been used to elucidate modified migration behavior following treatment with target complexes and light. Increased interaction with thymine-containing DNA in particular has been observed by CGE, suggesting a central role for thymine in DNA binding. In addition, polymerase chain reaction (PCR) followed by agarose gel electrophoresis has been used to evaluate the effects of TM complexes on replication for DNA vectors ranging from 128-144 bp. In these systems, we observe that irradiation of DNA in the presence of Cr(III) complexes results in a significant disruption of replication as well as the formation of DNA adducts, dependent on ligand identity/lability. Microcapillary CGE studies have provided verification of DNA damage and reduced replication efficiency suggested by agarose gels, but in a much more convenient and quantitative format with enhanced throughput.

Keywords: Bioanalytical, Capillary Electrophoresis, Electrophoresis
Application Code: Biomedical
Methodology Code: Capillary Electrophoresis
Since the initial discovery of cisplatin as an effective DNA chemotherapeutic, there has been interest in identifying biologically active transition metal complexes with increased selectivity and decreased toxicity. In recent years our lab has synthesized and characterized a variety of Cr(III) complexes in the form of [Cr(diimine)][sup]3+[/sup] or [Cr(diimine)][sup]2+[X[sub]2[/sub]][sup]3+[/sup], resulting in a range of enantiomeric substances with the potential to serve as DNA photocleavage agents. In supporting this effort, chiral capillary electrophoresis (CCE) has proven to be a convenient and effective tool to assess both chirality and product purity in a single run while using minimal sample. In addition to confirming the successful synthesis of the target complex, in some cases CCE can further be used to investigate the extent of interaction with DNA. Owing to the wide range of diimine ligands utilized, numerous chiral additives have been investigated to achieve separation, including the tartrate additives antimonyl-[i]d[/i]-tartrate and dibenzoyl-L-tartrate, both of which preferentially complex the [lambda] isomer. For example, compounds containing smaller diimines (e.g., 1,10-phenanthroline) demonstrate superior resolution with antimonyl tartrate, whereas larger, hydrophobic ligands that strongly intercalate DNA (e.g., dipyridophenazine) are best resolved using dibenzoyl tartrate. In addition to tartrate salts, sulfated cyclodextrins and a range of other amino-acid based derivatives have been shown to be useful for CE separation of chiral transition metal complexes.

Keywords: Bioanalytical, Capillary Electrophoresis, Chiral, Chiral Separations
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
**Peroxynitrite (ONOO-)** is a strongly oxidizing species that is produced in vivo by the reaction of excess superoxide (O2-) and nitric oxide (NO-). Peroxynitrite generation in vivo has been linked to cancer, as well as cardiovascular and neurodegenerative diseases. ONOO- has a very short half-life (~1 sec under physiological conditions), making its direct detection difficult. Therefore, this research is focused on the development of an indirect method for monitoring ONOO-production in macrophage cells using a novel fluorescent probe, HKGreen-3. HKGreen-3 has been shown to be selective towards ONOO-. Initial studies on the effect of pH on the yield of the fluorescent product was investigated. Due to the generation of side products during the reaction, a separation method was necessary for accurate quantitation of the desired product. This was accomplished using microchip electrophoresis with laser induced fluorescence detection. In order to obtain optimum resolution between HKGreen-3, side products, and the peroxynitrite product, both normal and reverse polarity electrophoretic separations were studied while varying surfactant concentrations. The use of reverse polarity only resulted in an increase of all migration times compared to normal polarity. After optimization was complete, this method was used to detect the ONOO-specific product in macrophage cells. Current studies are focused on the separation and detection of peroxynitrite in macrophages stimulated with lipopolysaccharide and phorbol-12 myristate acetate to produce NO- and O2-, respectively.

1 Peng T.; Yang D. 2010, Vol.12, No. 21, pp4932-4935

**Keywords:** Electrophoresis, Fluorescence, Lab-on-a-Chip/Microfluidics, Method Development

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
As a fatal, neurodegenerative movement disorder for which there is no cure and no effective treatment, research of Huntington’s disease is crucial. Clinical depression, which is often identified well before a definitive diagnosis of HD is made, is a common problem in patients. Serotonin is a key neurotransmitter implicated in the occurrence of depression; despite this fact, to our knowledge, investigations of the ability of neurons to release serotonin have not yet been published in the literature. The substantianigrareticulata (SNr) is richly innervated with serotonin-releasing terminals, and is thought to play a large role in movement, as well as reward. In this work, fast-scan cyclic voltammetry (FSCV) at carbon-fiber microelectrodes was used to compare serotonin release in acutely dissociated brain slices from wild-type and R6/2 Huntington’s disease model mice. An electrochemical waveform, designed specifically to increase the sensitivity of the electrode for serotonin while decreasing sensitivity for dopamine, was used to quantify serotonin release in the substantianigrareticulata (SNr). Using this method, we were able to positively identify and quantify serotonin release. Our data reveal that release is sharply diminished in 12-week-old R6/2 mice (18 ± 3.4% of WT), while it is relatively unaffected at 6 weeks of age (61 ± 9.8% of WT). In summary, this study shows a link between serotonin release and Huntington’s disease, which warrants further investigation of the mechanisms by which serotonin release is impaired in R6/2 mice.

**Keywords:** Electrochemistry, Microelectrode, Neurochemistry, Voltammetry

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
The use of analytical instrumentation to study differentiable human scent is an area of great forensic interest. Not only can the identification of individual scent profiles validate canine evidence in criminal investigations, but it can also determine a scent-based chemical fingerprint. Human scent comes from bacteria, eccrine and sebaceous gland excretions, and volatile organic compounds (VOCs), which are emitted from the dead epithelial cells of the skin. These VOCs can be present in different concentrations and combinations from person to person, making them important in determining differentiable scent profiles. Our goal was to study the VOCs in scent profiles and investigate the possible effects that variables such as age and sex have on human scent. Fifty samples taken from the unwashed hands of men and women aged 1 to 95 were studied. Samples were sealed in glass jars for 18-24 hours to allow the VOCs to concentrate in the headspace. Headspace cryogenic preconcentration and GC/MS were then used to analyze the resulting VOCs. The relative amount of ethanol was found to vary widely from sample to sample, so this compound was investigated as a potential marker for age and/or sex. Among the fifty subjects, samples given by young boys under the age of 16 were found to have the highest amounts of ethanol of any age or gender group. The amount of ethanol also decreased as the age group of the male donors increased. Conversely, samples given by young girls under the age of 16 contained low amounts of ethanol, with a general increasing trend in ethanol abundance with increasing female age groups. Although only a limited sample size has been studied, these data might suggest that both age and gender have an effect on the human scent profile, and that the abundance of certain VOCs within a profile might change over the course of a lifetime.

Keywords: Forensics, GC-MS, Quadrupole MS, Volatile Organic Compounds
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography/Mass Spectrometry
Understanding the Scott Test for Detection of Cocaine

The Scott Test, developed by Scott in 1973, has been modified in the study to improve the sensitivity towards the detection of cocaine. Identification of illegal substances using presumptive color tests is a technique widely employed by forensic laboratories and law enforcement agencies. Presumptive color test reagents are readily available, easy to use, and produce fast results, making them ideal for the use in field, in laboratory, or in the home. According to a survey performed by Carol L. O’Neal et al., more than 90% of the Drug Enforcement Administration laboratories frequently use Scott reagent for cocaine and Marquis reagent for other controlled substances. There is an increase in the worldwide consumption of cocaine in recent years. Although the Scott test has been around for almost four decades, there were only a few research papers available and they all have a limited explanation on how this test works. The theory behind the Scott test described that cobalt thiocyanate or primarily a cobalt ion forms a complex with the cocaine molecule, causing color to change from pink to blue.

Current study was designed to investigate the effects of many variables in the presumptive color test formulation to the sensitivity of detection towards cocaine. Using the factorial experimental design, the study will investigate the effect of cobalt concentration, solvent, ratio between cobalt ion and thiocyanate, glycerin concentration, presence of hydrochloric acid, mixing sequence, and pH. The study utilizes spectrophotometric technique to compare the responses of different circumstances for a 200-microgram cocaine sample. This study will gain an initial understanding on how Scott test works in different circumstances and will point future research directions to elucidate the false results caused by other interfering chemical species. This presentation will also make the users of Scott test aware of the possible cause of false positive and false negative.

Keywords: Chemical, Forensics
Application Code: Homeland Security/Forensics
Methodology Code: Chemical Methods
In vivo, insulin oscillations are found with a period of ~5 min. This oscillatory insulin signature is due to synchronization of millions of islets of Langerhans. Synchronization may occur through entrainment from in vivo glucose oscillations. Although intracellular [Ca2+] oscillations, which have been used as mimics of insulin oscillations, have been entrained to oscillatory glucose levels, there have been few demonstrations of direct entrainment of insulin secretion. To achieve this goal, a microfluidic system was developed that was capable of measuring temporally resolved insulin secretion from islets and was coupled with a gravity driven perfusion system to test if glucose waveforms can entrain insulin oscillations.

Gravity-driven flow through flow splitting channels was used to generate and deliver complex glucose waveforms to the islet chamber. This system was able to deliver sinusoidal waveforms with a period as low as 1.15 min with minimal peak broadening. Islet chamber dimensions and perfusion flow rate were adjusted for efficient sampling from single or groups of islets. Insulin secreted from islets was electrophoretically sampled and measured every 10 s with an online competitive immunoassay. This assay showed good reproducibility of the B/F with RSDs <4% and a detection limit of 2 nM.

Results showed insulin secretion from single islets was oscillatory with ~5-min periods. Both single and groups of islets were entrained to glucose sine waveforms. In addition to examining entrainment, this system will be useful for further studies of insulin secretion mechanisms and drug testing.

This work was supported by NIH grant DK080714.
Clothing fibers can potentially be used as crime scene evidence if the fibers can be compared with their source. Current methods, including FTIR, are able to distinguish the color and type of fiber, but cannot tell similar fibers apart. Isotope ratio mass spectrometry (IRMS) can be used to compare and differentiate these fibers based on their unique isotope ratios even between similar types. Past work has found different cotton clothing items to be statistically differentiable from each other using the carbon, hydrogen, and oxygen isotope ratios of the threads. In this research, we investigated if silks and wools obtained in different years and with different weave patterns were differentiable. Stained cotton was also investigated to determine if normal stains change the observed isotope ratios. It was found that all the silk and wool samples were statistically differentiable from each other with 95% confidence and that grass stains, dirt stains, and washing did not significantly affect the isotope ratios of cotton.
In actual forensic cases, powder residue inside empty plastic drug packaging may be the only available indication of drug presence. However, residue contains only trace amounts of drug, especially if “cut” or adulterated with other substances. An analytical method was sought to determine whether package residue could easily be collected and analyzed via a simple rinsing process, and to assess whether the sample would contain enough drug to provide reliable evidence of drug possession. Five abused drugs and nine common adulterants were selected. Samples containing 10% drug were created for each drug-adulterant combination. Each sample was placed in plastic packaging, with the bags subsequently emptied. The bags were then rinsed with water in order to collect any drug residue remaining in the package. Liquid samples were analyzed via Liquid Chromatography-Mass Spectrometry (LC-MS). Extracted ion monitoring was used to isolate the signal from the drug of interest to assess relative presence in the samples. Results showed that all five drugs were consistently detected in residue samples of adulterated drug containing 10% drug by mass.

A more quantitative analysis of methamphetamine, a highly abused drug, was then performed by making methamphetamine samples for each adulterant with drug amounts ranging from 4% to 0.1%. R\(^2\) values, limit of detection (LOD), and limit of quantification (LOQ) values were calculated for methamphetamine samples. Results showed that the method is capable of detecting methamphetamine in bag residues at levels approximately 36 to 755 times lower, depending on the adulterant, than typically found in street samples.

Keywords: Drugs, Forensics, Liquid Chromatography/Mass Spectroscopy, Trace Analysis
Application Code: Homeland Security/Forensics
Methodology Code: Liquid Chromatography/Mass Spectrometry
Proteins perform a multitude of functions in biological life and are of vital importance when analyzing cell behavior. This study was performed in collaboration with several institutions in which proteomic techniques were used to identify proteins expressed in *Tetrahymena thermophila* under various conditions and in specific structures. *T. thermophila* is a single-celled freshwater ciliate protozoan. It is a model organism, providing insight into cellular workings that can be extrapolated onto other species. Proteins were extracted from the organism and separated using SDS-PAGE gel electrophoresis. The protein bands were excised from the gel and digested with trypsin, with the resulting tryptic peptides purified with C18 pipette tips. Samples were analyzed using MALDI-TOF/TOF mass spectrometry. Tryptic peptides were further fragmented using tandem mass spectrometry techniques to allow sequence information to be obtained. These mass spectra were compared and matched with theoretical *T. thermophila* protein amino acid sequences using the MASCOT search database.

**Keywords:** Bioanalytical, Mass Spectrometry, Proteomics, Tandem Mass Spec

**Application Code:** Genomics, Proteomics and Other 'Omics

**Methodology Code:** Mass Spectrometry
Developing effective assays to easily measure molecular activity in cells is highly relevant for studying signaling pathways and identifying novel compounds for drug discovery. Here, we introduce a rapid, label-free analytical assay for measuring endogenous enzymatic activity. Conventional methods for measuring enzymatic events rely on time-consuming sample preparation including centrifugation, lysis, and lysate purification. More importantly, these conditions may lead to sample loss or decreased enzymatic activity thus limiting the validity of the readout. While cell-based assays have been developed as an alternative to conventional methods, these approaches utilize antibody capturing and fluorescence labeling, and therefore can only offer an indirect measurement. In this work, we develop a novel, label-free enzyme assay for simultaneous cell culture and lysate analysis that overcomes the inherent limitations of conventional and cell-based assays. This approach combines surface chemistry amenable for cell culture, along with self-assembled monolayer laser desorption/ionization mass spectrometry (SAMDI-MS), thus enabling cell culture and analysis on the same monolayer-coated surface. The surface chemistry was tailored to display a cell binding ligands (RGD) and reporter peptides bearing a target specific sequences. Using this approach, we simultaneously measured phosphatase and caspase-3 activity by in-situ lysis of a small population of cells cultured on monolayers and thus demonstrated that multiple enzymatic activities can be detected on the same monolayer. Furthermore, having established parameters to perform cell culture and obtain lysate analysis, we utilized the high-throughput capabilities of SAMDI-MS to screen a small molecule library and identify compounds that modulate phosphatase activity.

Keywords: Bioanalytical, High Throughput Chemical Analysis, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
According to the CDC, approximately half of the world's population lives in an area where they are in danger of being infected with the parasite Plasmodium falciparum which causes Malaria. More effective treatment of the disease would be greatly aided by a simple, rapid diagnostic test. This project details the development of a urine-based test for d-lactate which could be deployed in either paper or plastic microfluidic devices. D-lactate is produced at very high levels by the Plasmodium merozoites which are undergoing a very high rate of glycolysis and detoxify the resulting methylglyoxal using the methylglyoxylase pathway which results in d-lactate. Our assay exploits the selectivity of d-lactate dehydrogenase and a colorimetric or fluorometric detection reaction. We have found that this assay gives excellent selectivity and sensitivity at all relevant physiological levels. The presented work will detail our efforts to stabilize the biomolecules and implement this assay on both plastic and paper microfluidic devices.

Keywords: Biomedical, Capillary Electrophoresis, Fluorescence, Lab-on-a-Chip/Microfluidics
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Abstract Text
Organic contamination of the groundwater is a major area of interest in agricultural and industrial sites as well as lands involved in hydrofracking. The goal of this project is to develop an automated microfluidic device which can preconcentrate, separate and then detect low abundance organic contaminants in water. For the preconcentration step, we are using magnetic Osorb beads from ABS materials which are embedded with magnetite. We have determined optimum pre-concentration volumes and releasing solutions for a number of analytes. Separation results will also be presented along with prototype centrifugal chip designs.

Keywords: Environmental/Water, Extraction, Lab-on-a-Chip/Microfluidics, Petrochemical
Application Code: Environmental
Methodology Code: Microfluidics/Lab-on-a-Chip
Development of Portable Microplate Reader Using Organic Light-Emitting Diodes and Photodiodes

Enzyme-linked immunosorbent assay (ELISA) is one of the most powerful methods to determine a protein using antigen-antibody reaction and enzyme reaction. ELISA is widely used for analyzing biological samples because of its high sensitivity and selectivity. A 96-well microtiter plate and a microplate reader are commonly used for conventional ELISA since a large number of analyte can be tested simultaneously by using the plate and the plate reader. However, to perform ELISA in the field seems to be difficult because of the size and cost of the microplate reader. To solve this problem, we developed a novel microplate reader with nine fluorescence detection points using laboratory made organic light-emitting diodes and photodiodes as light source and photo detector, respectively. The size and weight of the microplate reader was 50 x 50 x 200 mm and 95 g, respectively. A 9-well microtiter plate for the developed microplate reader was also fabricated. The bottom of the well was made of pure PDMS to transmit the fluorescence from the sample solution in the well to the photodiode, whereas the wall of the well was made of PDMS containing 5 % activated carbon to prevent cross talk with adjoining well. The fluorescence detection system consisting of the 9-well microtiter plate and the plate reader was applied to sandwich-ELISA for the rapid determination of IgA in human saliva, a marker for human stress. The detection limit (3σ) for IgA was estimated to 9.1 ng/mL and the sensitivity of detection was sufficient to evaluate human stress, since human saliva contains 110-220 g/mL of IgA. The sensitivity of the developed microplate reader was comparable to that of commercially available microplate reader. Compared with conventional ELISA using a 96-well microtiter plate, the processing time could be reduced to half and the reagent volume could be cut to one third, respectively. In addition, the developed system was successfully applied to the antibody test on rubeola.

Keywords: Bioanalytical, Detection

Application Code: Bioanalytical

Methodology Code: Sensors
ChemSpider is an online database of over 30 million chemical compounds sourced from over 500 different sources including government laboratories, chemical vendors, public resources and publications. Developed with the intention of building community for chemists ChemSpider allows its users to deposit data including structures, properties, links to external resources and various forms of spectral data. Over the past few years ChemSpider has aggregated almost 20000 high quality NMR and IR spectra and continues to expand as the community deposits additional types of data. The majority of spectral data is licensed as Open Data allowing it to be downloaded and reused in presentations, lesson plans and for teaching purposes. This poster will present our existing technology and our plans to host a million spectra in our developing online data repository.

Keywords: Data Analysis, Database, Informatics, NMR
Application Code: General Interest
Methodology Code: Education/Teaching
We have developed a new method of electrochemical cytometry to measure the total content of single neurotransmitter vesicles and we have compared this to exocytotic release to measure the extent of partial release. We have carried out vesicle impact experiments at electrode microarrays and used this to model the vesicle diffusion coefficient. The electrochemical response to single adrenal chromaffin vesicles filled with hormone transmitters as they impact a 33-um diameter disk-shaped carbon electrode will be shown. The vesicles appear to adsorb onto the electrode surface and sequentially spread out over the electrode surface trapping their contents against the electrode. These contents are then oxidized and a peak results for each vesicle that bursts. A large number of current transients can be observed if the concentration of vesicles is high relative to the area of the electrode. Comparison of the contents of these biological vesicles to the release of catecholamine from single cells supports the concept that only a fraction of transmitter is released during exocytosis. Additionally, substructure of the vesicles is apparent in the shape of the current transients. Finally, we have succeeded in carrying out vesicle impact electrochemical cytometry inside living cells. Here we have been able to compare directly the contents of vesicles to the amount released from the cell.
Some thirty-five years ago, the electrochemical detection of neurotransmitters in vivo became possible with the miniaturization of electrodes to the micrometer scale. Over the past two decades, advances in electroanalytical chemistry have enabled selective quantification of subsecond fluctuations of electroactive molecules such as dopamine, a neurotransmitter that is important in movement and reward processes. Studies that utilize fast-scan cyclic voltammetry, an electroanalytical approach that combines selectivity with sensitivity to report on rapid dopamine fluctuations at bare carbon electrodes in the brain, are of broad interest to both cellular and behavioral neuroscientists, and are truly advancing our understanding of goal-directed behavior. However, the voltammetric approach that has proven to be so powerful has largely been used only to study dopamine, and the dynamic fluctuations of countless classes of molecules in the brain remain uncharacterized. This talk will discuss the development of innovative new analytical tools aimed at improving the detection of dopamine and enabling real-time electrochemical measurements of additional classes of molecules in the brain. Novel electrode materials, electrochemical waveforms, and approaches to the voltammetric detection of hydrogen peroxide at enzyme-modified electrodes will be discussed.

This work is supported by the NIH (R01NS076772, R03DA027969) and the NSF (CAREER CHE7151264).

Keywords: Biosensors, Magnetic Resonance, Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
In this talk, I will present our recent research on nanoscale electrochemical imaging with fluorescence-enabled electrochemical microscopy (FEEM). FEEM is a new electrochemical technique developed in our laboratory to image individual redox events with exceedingly high spatial and temporal resolutions. The central idea of FEEM is the coupling between a redox reaction of interest and a fluorogenic reaction on a closed bipolar electrode. This allows one to use fluorescence to monitor conventional electrochemical processes. Our group has developed very large electrochemical arrays containing millions of ultramicroelectrodes (UMEs) and nanoelectrodes and used these arrays to image biological events. Here, I will describe our recent progress on the use of such arrays to image neuronal activity from single neuronal cells and their network.

Keywords: Electrochemistry, Imaging, Neurochemistry
This presentation will describe new motion-based bioassays based on changes in the speed or distance of nanomotors induced by biomolecular interactions or on the selective capture, sorting and transport of target biomolecules. Different motion transduction schemes will be illustrated for monitoring a wide range of biomolecular interactions of nucleic acids, proteins, bacteria or cancer cells. The resulting assays add new and rich dimensions of analytical information and offer remarkable sensitivity, coupled with simplicity, speed and low costs. Microengines functionalized with different receptors will be shown to capture selectively and transporting target DNA and cancer cells from raw complex body fluids. New microengines with a ‘built-in’ recognition capability will also be discussed. The latter obviated the need for the receptor immobilization. The greatly improved capabilities of chemically-powered artificial nanomotors could pave the way to exciting and important bioanalytical applications and to sophisticated nanoscale and microchip devices.

References:
3. S. Balasubramanian et al., Angew Chemie Ind Ed. 50(2011)4161.

Keywords: Nanotechnology, Robotics, Separation Sciences
Application Code: Biomedical
Methodology Code: Separation Sciences
In the nervous system of all animals studied to date, including mammals, D-amino acids are present. As examples, D-serine, D-aspartate and D-alanine are present at high levels in specific brain and endocrine regions, although their functions are not well understood. Because neurochemistry is well conserved across metazoan life, we find these molecules in a range of animals, and are studying their formation and function in cell to cell signaling. We are developing a range of analytical tools to characterize the chemical content of small brain regions, even down to individual neurons. Using capillary electrophoresis with laser induced fluorescence and mass spectrometry detection, we have determined which neurons synthesize D-Asp, and have measured the formation, transport and release of D-Asp in a stimulation dependent manner; we have even characterized the enzyme responsible for D-Asp formation. We are currently measuring the presence of other D-amino acids in neurons and endocrine structures and adapting sampling approaches to measure their activity-dependent release. Besides amino acids, neuropeptides are an important category of neuromodulatory compound. While proteins (and thus, their peptide products) are ribosomally translated using only L-amino acids, D-amino containing-peptides (DAACPs) do exist, although are difficult to characterize using mass spectrometry alone because the modification does not have an associated change in mass. We have developed a series of mass spectrometry approaches to identify DAACPs from complex mixtures of peptides. Our DAACP discovery funnel has already identified several candidate neuropeptides and more appear likely. Thus, this enigmatic post-translational modification to a peptide may be more common than previously thought.

Keywords: Capillary Electrophoresis, Mass Spectrometry, Neurochemistry

Application Code: Neurochemistry

Methodology Code: Separation Sciences
Measurements of the phase of femtosecond nonlinear optical signal fields opened up the possibility of measuring optical frequency Two-Dimensional Fourier Transform (2DFT) spectra that are in some ways analogous to 2DFT NMR spectra, which correlate pairs of frequencies from the same molecule. Since initial demonstration and development at optical frequencies, femtosecond 2DFT spectroscopy has been extended down to the THz region, up to the deep ultraviolet, and widely used to study gases, liquids, solids, glasses and proteins. There is now a quantitative theory for this nonlinear spectroscopy in three frequency dimensions that generalizes the one-dimensional Beer-Lambert law of linear spectroscopy. An overview of the principles, capabilities, and prospects for femtosecond 2DFT spectroscopy will be followed by discussion of recent results on molecules and photosynthetic proteins, where the usual adiabatic picture of fast electrons and slow vibrations fails for all accessible coordinates.
Ultrafast coherent two-dimensional infrared (2D IR) spectroscopy is a powerful approach for obtaining structural features and equilibrium dynamics of peptides and proteins. In this talk, I will first present the results of 2D IR experiments on oligopeptides that serve as a paradigm for studies of backbone conformations in proteins. Using multiple pulse sequences and polarization configurations as well as strategic isotope substitutions, we demonstrate that 2D IR can provide diagnostic cross-peak patterns for distinguishing different secondary structure, probe the onset of secondary structure, and reveal complex vibrational couplings between the amide-I and amide-II modes. Experimental spectra are compared to simulations based on nonlinear response theory, vibrational eigenstates and couplings derived from DFT-optimized structures, and trajectories from molecular dynamics (MD) simulations. Our results show that 2D IR can provide a large set of spectral constraints that are useful for determining peptide structure and testing theoretical models. In the second part of my talk, I will describe the use of waiting-time dependent 2D IR to study ultrafast molecular dynamics, such as vibrational relaxation, energy and coherence transfer, frequency fluctuation correlation, and chemical exchange. Recent applications on peptide-membrane interaction, conformational exchange, and hydrogen bond dynamics will be discussed. Experimental results are compared with those from MD simulations, DFT calculations, and stochastic simulation.
Mechanisms that govern light-induced chemical reactions are of interest to a broad scientific community of physicists, chemists, and biologists. Time-resolved resonance Raman spectroscopies are among the most indispensable tools used in such investigations, because resonance enhancement enables studies of dilute solutions and active sites in complex systems. Sensitivity of time-resolved resonance Raman spectroscopies to dynamics on the 100-fs time-scale requires use of a stimulated (rather than spontaneous) Raman probe. Such techniques are referred to as femtosecond stimulated Raman spectroscopies (FSRS). This talk will introduce two new approaches to FSRS. In both methods, five incident laser beams are used to eliminate most of the background that challenges signal detection in the traditional three-beam FSRS geometry; data acquisition rates and signal-to-noise ratios are improved by 1-2 orders of magnitude using five beams. Applications to photodissociation processes in triiodide and oxymyoglobin will be discussed.

Keywords: Raman, Ultra Fast Spectroscopy, Vibrational Spectroscopy
Application Code: General Interest
Methodology Code: Molecular Spectroscopy
Coherent Multidimensional Analogues of IR and Vis/UV Absorption, Emission, Raman, and Multiphoton Spectroscopies and Their Applications to Molecular and Materials Spectroscopy

Analytical spectroscopy is dominated by 1D UV/vis, infrared, fluorescence, and Raman spectroscopies but NMR has a rich variety of methods based upon multiple spin coherences. Successive pulses excite multiple spin states on time scales that are shorter than the loss of quantum mechanical phase. This talk will describe a new coherent family of coherent electronic and vibrational spectroscopies that are analogues of NMR methods and an enhancement of traditional incoherent spectroscopies. The family is based on using separately tunable lasers to excite a mixture of electronic and/or vibrational quantum states called a multiple quantum coherence (MQC). Since the lasers are temporally and spatially coherent, so also is the mixture of quantum states. Pairs of states within the MQC emit cooperatively throughout the excitation region in beams that depend on the frequency differences between the pairs. The method is multidimensional and measures the resonance enhancements as a function of the excitation frequencies, time delays between excitation pulses, and the output frequency. Two dimensional cross-sections through the multidimensional variable space create spectra that define the couplings between vibrational and/or electronic states and the coherent and incoherent dynamics. The methods share many of the advantages of multidimensional NMR methods. The multidimensional capabilities provide high selectivity that permit analytical measurements on complex samples. The coupling requirement eliminates much of the spectral congestion in complex samples because only spectral features that are coupled will appear in the spectra. The ultrafast excitation pulses provide temporal resolution on femtosecond time scales so the coherent dynamics can be measured in addition to the slower incoherent population dynamics. Example applications will illustrate the capabilities of these methods and how they can be used for analytical spectroscopy and materials chemistry.

Keywords: Laser, Molecular Spectroscopy, Ultra Fast Spectroscopy, Vibrational Spectroscopy

Application Code: Other
Methodology Code: Molecular Spectroscopy
Coherent Spectroscopy and Coherent Control All Across the Spectrum

It is now possible to conduct coherent spectroscopy across an amazing swath of the electromagnetic spectrum, from radiofrequency to x-ray ranges. The ability to generate tailored coherent fields throughout the spectrum is exploited to control molecular and collective responses as well as to measure them. Recently developed capabilities in two spectral regions will be presented. First, terahertz-frequency fields can now be generated that are strong enough to generate nonlinear responses of molecules and materials. Their use for coherent spectroscopy and control of rotations of polar molecules, yielding transient orientation of molecular dipoles, and their use to drive crystalline-phase electronic and structural phase transitions that can be monitored by time-resolved x-ray diffraction, will be illustrated. The strong THz fields are themselves the products of coherent control, generated by spatially and temporally shaped optical pulses that drive polar crystal lattice vibrations that radiate in the THz range. Spatial and temporal shaping of optical fields is used for fully coherent multidimensional optical spectroscopy and control of collective electronic as well as vibrational responses. Studies of correlated exciton and multi-exciton states including exciton-polariton Bose-Einstein condensates will be described.

Keywords: Materials Science, Molecular Spectroscopy, Spectroscopy, Ultra Fast Spectroscopy
Application Code: Materials Science
Methodology Code: Other
## Interfacial Complexes in Spent Nuclear Fuel Reprocessing Systems

Compared to other condensed phase media, very little is known about the inhomogeneous environment at the interface between two immiscible liquids in spent nuclear fuel reprocessing. It is expected that unique structural and dynamical properties exist in this interfacial region. However, due to the small size of the region which is buried between the two immiscible liquids, it is challenging to experimentally probe these properties. Many studies have utilized spectroscopic and X-ray techniques to probe the interfacial chemistry of two immiscible liquids. While these techniques have greatly enhanced our knowledge of interfacial phenomena in the past decade, they are unable to directly probe many properties. At the University of Utah, we are using modern, biphasic analysis techniques including biphasic electrospray ionization, biphasic atmospheric pressure chemical ionization, and biphasic calorimetry to characterize the thermodynamics, kinetics, hydration, and ion transfer of f-element cations at the interface of immiscible liquids.

### Keywords:
- Characterization
- Mass Spectrometry
- Nuclear Analytical Applications
- Nuclear Energy

### Application Code:
- Nuclear

### Methodology Code:
- Other
Analytical sensors are needed to monitor the composition of radioactive wastes during storage and processing. Complex salt solutions, solids and gases in nuclear waste storage tanks need to be monitor constantly to ensure the integrity of waste tanks and to safeguard the processing and disposal of nuclear wastes. Raman spectroscopy is an ideal analytical tool for waste tank analysis because it can be used for identification and quantitation of the chemicals in the waste. Advances in Raman technology, both in compact instrumentation using solid-state lasers, charge coupled device detectors and fiber optic sampling, allow the application of Raman as a remote monitoring technique. Fiber optic probes connected to the Raman instrument can be delivered inside waste tanks for remote characterization of waste materials. A Raman/turbidity sensor, Raman telescope probe and Raman gas cell have been developed for nuclear waste monitoring applications. The development of these Raman sensors will be presented.

Keywords: Laser, Sensors, Spectroscopy
Application Code: Other
Methodology Code: Vibrational Spectroscopy
The goal of this work is the development of a sensor for 99Tc that is applicable for characterizing and monitoring for the presence of technetium in the environment and in nuclear waste. The dominant form of Tc species in the vadose zone and ground water at Hanford and other US-DOE sites is pertechnetate, TcO4-, while lower oxidation states are believed to be present within select nuclear waste storage tanks. The sensor consists of an innovative fluorescence-based spectroelectrochemical configuration that we have developed by combining the following elements: spectroscopy, electrochemistry, device fabrication, thin film technology, and synthetic inorganic chemistry. The spectroelectrochemical sensor has been demonstrated on a variety of chemical systems including authentic radioactive waste samples. In pursuit of our project goals, we have discovered and report the first examples of excited-state luminescence from technetium complexes. We have examined a series of trans-dioxo complexes of Tc(V), a Tc(I/II) phosphine complex, and technetium(I)-tricarbonyl complexes and compare their respective photophysical properties with the corresponding rhenium analogues. In a recent application, we have focused on making thin chemically-selective films for sensing radioactive technetium compounds and in this effort have developed a fluorescence-based spectroelectrochemical sensor. Characterization of the technetium excited states as well as application of the respective chromophores for use in a spectroelectrochemical sensor for technetium will be discussed.

Keywords: Chemical, Electrochemistry, Nuclear Analytical Applications, Spectroelectrochemistry
Application Code: Nuclear
Methodology Code: Fluorescence/Luminescence
Tritium is a radioactive beta decay material which is required for fusion research. It has a half-life of 12.3 years that translates to approximately 50,000 curies in just 6 grams of material in its T2 gas form. It can readily undergo exchange reactions with protium sources such as hydrocarbons and water. Even the hydrogen out-gassing from high vacuum systems of a mass spectrometer will cause significant analysis constraints. Tritium turns fluorocarbons to thick syrup and the activity level can charge up detectors. As a result instrumental analysis must handle several chemical and material constraints. Examples from experiences at the Savannah River National Laboratory (SRNL) will be discussed in the presentation. For example, instruments need to replace o-ring seals with metal vacuum seals and instruments need to be able to work at negative pressures in closed systems inside of secondary inert containment glove-boxes to limit exposure and other risks. Chemical analysis becomes a challenge. For example a simple methane analysis can go from CH4 to CHD3, CH2D2, CH3D, CD4, ...CH3T, ...CHDT2, ...CDT3, ...CT4 dependent on the HDT mix while still giving a single GC peak for methane, but variant spectra in the IR and Raman, and complex mass spectroscopy patterns that require high resolution systems. Passivated high resolution mass spectrometers with minimal residual hydrogen background are required for some analysis. To find alternative analysis solutions SRNL has built a Tritium Instrumentation Demonstration System to test and evaluate analytical instruments that complement mass spectrometry. These systems are totally contained and connected to tritium recapturing systems and supported with trace level ion chambers and evacuation processes.

**Keywords:** Energy, Nuclear Analytical Applications

**Application Code:** Nuclear

**Methodology Code:** Process Analytical Techniques
Monitoring and control of actinide oxidation states is critical to maintain safety and efficiency in nuclear materials processing. The absorption spectra of the actinides are sensitive to oxidation state, solution acidity (nitric acid), and temperature, making it difficult to establish an accurate universal regression model for all possible process conditions. Furthermore, for plutonium, oxidation and/or disproportionation will naturally occur at some conditions, meaning that stable standards cannot be made without introducing chemical stabilizers that can influence the calibration. Here, we describe two approaches to creating analytical methods for the measurement of chemically unstable forms of Pu. The first is a process absorption spectroscopy method whereby spectra are classified into one of several regimes. Localized calibrations are based on spectra taken as the solutions naturally evolve – the total Pu concentration remains constant but the relative amounts of different oxidation states vary. The relative concentrations of these species are determined by principal components analysis, the results are used as the basis for subsequent predictive models. Because this approach is resource-intensive, we have incorporated calibration transfer schemes to allow a single calibration to be ported to multiple instruments. The implementation of these schemes into a Pu processing facility at Savannah River Site is described. The second approach is to use spectroelectrochemistry to control the Pu oxidation state at any acidity, again avoiding the use of chemical stabilizers. Ideally, complete control of the solution could be obtained. In practice, the range of conditions where spectroelectrochemistry can be performed may not be as large as initially hoped. We will describe the successes and apparent limitations of the method, as well as operational considerations for its use as a routine analytical method.

Keywords: Chemometrics, Nuclear Analytical Applications, Process Analytical Chemistry, UV-VIS Absorbance/Lu
Application Code: Nuclear
Methodology Code: UV/VIS
Antibody-drug conjugates (ADCs) harness the specificity of tumor-recognizing mAbs to deliver a cytotoxic agent directly to cancerous cells thus avoiding the systemic toxicity associated with standard chemotherapy. The potency of ADCs depends on the structural integrity of the mAb, the cytotoxic agent, and the conjoining linker. High resolution mass spectrometry (MS) has long been used to elucidate protein primary structure and, more recently, has become important in biopharmaceutical development where it is leveraged to characterize the structural integrity of biopharmaceutical products. We present here 2 case studies highlighting the application of MS in biopharmaceutical development to define the higher order structural integrity and micro-heterogeneity of ADCs. The first case study involves the application of hydrogen-deuterium exchange mass spectrometry to the direct comparison of the higher-order structure of a mAb and the corresponding ADC which is composed of the mAb with engineered cysteine residues in the Fc domain that are conjugated to pyrrolobenzodiazepine (PBD) dimer drug-linkers. Our findings indicated that there were no significant conformational differences between the mAb and the corresponding ADC. The second case study focuses on the application of MS to the analysis of thermally stressed mAbs and ADCs that are prepared as freeze-dried lyophilized cakes. We will present recent results showing that lyophilized proteins that undergo thermal stress can form covalent adducts with the buffer and excipient components present in the formulation.

Keywords: Biopharmaceutical, Liquid Chromatography/Mass Spectroscopy, Pharmaceutical, Protein
Application Code: Pharmaceutical
Methodology Code: Mass Spectrometry
Innovative Mass Spectrometric Characterization of Biopharmaceuticals

Improved Characterization of Antibody Biotherapeutics with Ultrahigh-Resolution Mass Spectrometry

Ultrahigh-resolution mass spectrometers in combination with platform/standardized methods offer significant improvements for the structural characterization of protein therapeutics, especially for monoclonal antibodies (mAbs). Specialized LC/MS-subunit analysis characterization methods were developed for mAbs that provide rapid, definitive confirmation of the light and heavy chain amino acid sequences and detection of posttranslational and storage-induced modifications at the subunit level, including deamidation. In addition, LC/MS-subunit analysis offers rapid de novo detection of potential sequence variants at the subunit level during early stages of process development. This approach has been advantageous for screening multiple cell clones for higher-level sequence variants prior to final clone selection.

Keywords: Biotechnology, Characterization, Liquid Chromatography/Mass Spectroscopy, Protein
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Constant analytical progress has resulted in better understanding at the molecular level of the attributes that are crucial for safety and efficacy of protein drugs. Parallel to these advances, regulatory agencies have recommended a Quality by Design (QbD) approach to integrate the knowledge of these critical quality attributes (CQAs) in drug manufacturing. A logical next step for the biopharmaceutical companies that have embraced QbD and CQA during process development would be the applications of these concepts for data-directed manufacturing and quality control associated with drug disposition. In this communication, we present the design of a MS-based approach for automated assessment of product quality attributes of therapeutic proteins. Our goal is to leverage the deep knowledge of CQAs acquired during research and development to provide feedback mechanisms for real-time control of drug manufacturing and a new paradigm for release testing. The approach is based on the detailed analysis of the drug by peptide mapping using a combination of high accuracy / high resolution MS data generated by Orbitrap technology and automated identification and quantification of CQAs with a dedicated quantitative software program. Application of these principles results in extensive sequence coverage of complex proteins and quantitative measurement of multiple attributes, such as glycoform distribution and amino acid modifications. Concrete application of this approach for quantitative analysis of monoclonal antibodies in a quality control environment aligns well with QbD principles by focusing on the attributes of the drug essential for function. Current results indicate that this knowledge-based, multi-attribute single method can be used for real-time assessment of drug quality during manufacturing and has the potential to replace several conventional electrophoretic and chromatographic methods currently used to release biologics.
Mass spectrometry has increasingly become an indispensable tool in biopharmaceutical process development, in addition to its common use in product characterization. Recent advances in instrument capability, both hardware and software, provide the required sensitivity, resolution, speed and dynamic range for detection of low level product related variants and process related impurities. A common approach employed is peptide mapping with reversed phase HPLC or UHPLC, with online LC-MS/MS analysis using data dependent acquisition.

Several specific areas of mass spectrometry application in biopharmaceutical development are included in this presentation. One is sequence variant analysis in support of cell line selection and cell culture process development. Low level, previously unknown amino acid substitutions due to either mutation or mistranslation are identified with the use of mass spectrometry. Recent studies on analytical approaches towards improved detection coverage and the use of appropriate assay control will be presented. A second area is the characterization of product color due to oxidation and advanced glycation end products modifications on lysine residues. These variants are highly heterogeneous in nature and are present at very low levels, both representing significant challenges in analytical technologies. Cell culture media and process conditions that yield reduced color modification have been identified. We describe in the third area recent advances in the use of mass spectrometry to complement the conventional immunoassay approaches for host cell protein (HCP) analysis. Analytical methodology and case studies will be presented for both the identification and quantification of HCP to support the optimization of purification process.

In summary, we demonstrate that mass spectrometry is a critical analytical tool for development of biopharmaceutical products that are safe, consistent, and well characterized.
Samples of purified biologics typically contain extremely low levels of sequence variants of the active pharmaceutical ingredient as well as host cell proteins. The reliable detection and identification of such molecules is greatly hindered by the presence of the active pharmaceutical ingredient. Nevertheless, the tremendous advances in mass spectrometry enable the identification and quantification of even these very low abundant proteins.

We present mass spectrometry-based approaches that enable a sensitive identification and quantification of low abundant protein species directly in samples containing large quantities of purified biologics. Potential pitfalls of such proteomic approaches and remedies to avoid misinterpretation of the results are discussed.

**Keywords:** Characterization, Mass Spectrometry, Proteomics, Quantitative

**Application Code:** Biomedical

**Methodology Code:** Mass Spectrometry
Nano-/quantum-biodevice is a piece of contrivance, equipment, machine, or component, which is created by the overlapping multidisciplinary activities associated with nano-/quantum-technology and biotechnology, intended for biological, medical, and clinical purposes. In this lecture, I will describe the development of nano-/quantum-biodevices for biomedical applications, including single cancer cell diagnosis for cancer metastasis, circulating tumor cell (CTC) detection by microfluidic devices, nanopillar devices for ultrafast analysis of genomic DNA and microRNA, nanopore devices for single DNA and microRNA sequencing, nanowire devices for exosome analysis, single-molecular epigenetic analysis, quantum switching in vivo imaging of iPS cells and stem cells, and quantum technology-based cancer theranostics. Euglena-based “biomimetic mechanical system” enables us to develop reliable circulating tumor cell (CTC) separation and detection technique for cancer metastasis diagnosis. Immunopillar devices realized the fast and low invasive “from blood to analysis” type biomarker detection of cancer with fM detection sensitivity within 2 min. Additionally, nanopillar devices give us ultrafast separation of DNA and microRNA within 60 µs and nanopillar-nanopore integrated nanobiodevice enables us ultrarfast single molecular DNA sequencing. Nanowire devices coupled with super-resolution optical microscopy are extremely useful to analyze exosomes from cancer cells and exosomal microRNA analysis. Quantum dots are applied to develop nanobiodevice for single cancer cell diagnosis, single molecular epigenetic analysis, quantum switching in vivo imaging for iPS cell and stem cell therapy and theranostic device for cancer.
A current focus of biological research is to quantify and image cellular processes in living subjects. To analyze such cellular processes, genetically-encoded reporters have been extensively used. The most common reporters are firefly luciferase, renilla luciferase, green fluorescent protein (GFP) and its variants with various spectral properties. Herein, novel design of split GFP and split luciferase will be described; the principle is based on reconstitution of the split-reporter fragments; The basic strategy of the reconstitution is to split a reporter protein into two non-active fragments that are fused to a pair of interacting proteins. The interaction between the two proteins brings the two fragments into close proximity, allowing reconstitution of an intact reporter protein. To demonstrate the usefulness of the reconstitution technology, we have applied the reporters for developing a genetic method to identify mitochondrial proteins and their localization, and imaging dynamics of endogenous mRNA in single living cells. We have recently used split-luciferase reporters with different spectral characteristics for GPCR-arrestin interactions in living subjects. We have developed another design of reporter proteins; a cyclic luciferase by protein splicing to monitor protease activities in living cells and mice. Herein, we will focus on recent advances in the imaging technologies with fluorescent and bioluminescent proteins.
Designing Mechanized Nanoparticles for Cancer Therapy and Diagnosis: Toward Developing Nanorobots

One of the challenges in cancer therapy and diagnosis is to develop novel nanomaterials that can carry out targeted, controlled release of anticancer drugs and siRNA as well as to enhance imaging and diagnosis. We have been developing mechanized nanoparticles based on mesoporous silica nanoparticles (MSNs) that contain thousands of pores where chemicals can be stored. MSNs can be surface modified to provide tumor targeting capability. Efficacy of camptothecin-loaded MSNs to inhibit tumor growth was demonstrated in animal model systems. Extensive analysis of safety and biocompatibility of this type of material was carried out.

We equip MSNs with nanovalves that can cap the pore opening with a bulky chemical group. By opening and closing nanovalves, it is possible to achieve controlled release of the content. One type of nanovalves responds to change in pH and opens in acidic pH conditions that are encountered inside the cell or in the tumor environment. Another type of nanovalve contains a disulfide bond so that the nanovalve can respond to reducing conditions inside the cell. We have also developed MSNs that respond to light. This system uses azobenzene, a photosensitive chemical that changes conformation in response to light exposure. We call this drug delivery system nanoimpeller that releases anticancer drugs in response to blue light. More recently, we have developed a second generation nanoimpeller by incorporating a two-photon responsive fluorophore. This enabled the use of red or near infrared red light that are tissue penetrating. Finally, we have developed MSNs that respond to magnetic field. This MSN contains iron oxide core that has super paramagnetic property. Exposure to oscillating magnetic field increases temperature of MSNs opening the nanovalve and releasing contents. Iron oxide core also provides MRI enhancing property. Thus, these MSNs have multiple features that are ideal for imaging and therapeutic purposes.

Keywords: Biomedical, Drugs
Application Code: Biomedical
Methodology Code: Chemical Methods
Discoveries at the nanoscale continue to affect all of us at the global and local scales. This is especially true for the next generation of biomaterials as their development demands improved imaging and analytical performance. Hitachi is responding to this challenge with its HT7700 and the EXALENS, a high resolution objective lens that sets the new performance standard for 40-120kV microscopy. By achieving a smaller spherical aberration coefficient, a lattice resolution of 1.4Å is demonstrated. EXALENS excels at high resolution transmission electron microscopy (TEM) imaging at low accelerating voltages with minimal beam damage, facilitating analyses of soft materials, carbon-based nanomaterials, polymers and catalysts. It is the superior choice for brightfield/darkfield scanning transmission electron microscopy (STEM), where thick biological specimens are imaged with reduced chromatic aberration. These improvements in electron optics, as well as recent developments in TEM instrumentation, have made it possible to study dynamic in-situ reactions of nanoparticles engineered for disease diagnosis and treatment.

Keywords: Bioanalytical, Biomedical, Microscopy, Nanotechnology

Application Code: Nanotechnology

Methodology Code: Microscopy
Analysis of the affinity and the binding between bio-molecules and between molecule and cell tends more and more towards label-free approaches. Biosensors and Biochips are developed worldwide to propose new, fast, cheap, reliable and multiplexed tools to meet these demands.

SPR Imaging is moving the molecular interaction analysis a step further by offering the multiplex, label-free and real time detection. However, biological samples often contain attomolar or femtomolar concentrations of biomolecules, which is below the detection limit of SPRi. It is not common to combine the SPR to nanotechnology investigation. The high flexibility of the SPRi instrument as well as the biosensor itself open the way to reach ultra low sensitivity measurements.

We present here the exploitation of the powerful approach of Surface Plasmon Resonance Imaging (SPRi) and the unique combination with nanomaterials leading to ultrasensitive level for biomarkers detection. During this presentation we will show you different applications which are promising especially to increase the progress of Health (diagnostic analysis and treatment).

Keywords: Bioanalytical, Biomedical, Biopharmaceutical, Biosensors
Application Code: Bioanalytical
Methodology Code: Other
Tandem mass spectrometry has proven to be an increasingly popular strategy for elucidating structural information from non-covalent complexes. Collisional-induced dissociation (CID) is frequently utilized for disassembly of protein complexes and analysis of the constituents. However obtaining complete sequence information from proteins within non-covalent complexes is challenging, typically requiring either several MSn events or higher-energy collisional dissociation. Ultraviolet photodissociation (UVPD) is a high energy fast ion activation/fragmentation method which affords rich sequence information. Here we successfully utilize UVPD as an effective MS/MS top-down strategy for characterization of native protein-ligand and protein-protein complexes as well as proteins with post-translational modifications. For example, characterization of protein ubiquitination, a post-translational modification that modulates protein degradation and cell cycle control, is challenging due to changes in the site of modification, the number of ubiquitins attached, and the linkages between ubiquitins. Ultraviolet photodissociation offers a promising strategy to assign the linkage type of polyubiquitin modifications.
Optimized Tandem MS for Biomedical Studies

The analysis of samples from biomedical sources presents multiple challenges: limited amounts of material, mixtures of components with closely related structures, labile structural modifications, temporal changes due to growth and development, aging, infection and disease, environmental exposures, etc. We are particularly interested in the qualitative and quantitative detection of protein post-translational modifications and glycoform distributions. Both LC/MS and MALDI-MS approaches are being applied to studies of cardiovascular, infectious and protein misfolding diseases, using high performance MS/MS and IMS/MS methods, in combination with various dissociation strategies.

Keywords: Biomedical, Carbohydrates, Mass Spectrometry, Protein
Application Code: Biomedical
Methodology Code: Mass Spectrometry
In this work, we combine immuno-laser capture microdissection (iLCM) and liquid chromatography mass spectrometry (MS) as a means to study proteins that are specific for pure cell subpopulations in complex tissues. CD24, as a cell surface marker for detecting pancreatic cancer stem cells (CSCs), is directly correlated with the development and metastasis of pancreatic cancer. An in-depth proteomic profiling method for frozen pancreatic CD24+ adenocarcinoma cells using iLCM and LC-MS/MS, compared with CD24- cells dissected from patient-matched normal adjacent tissues will be described. Around 40 nL tissue procured from each specimen, which corresponds to ~1.3 μg of protein material, was subjected to tandem MS analysis in triplicates. A total of 2665 proteins were identified, with 379 proteins in common that were significantly deregulated in CD24+ versus CD24- cells, with at least 2-fold change in expression. Ingenuity Pathway analysis (IPA) of these differentially expressed proteins further suggests significant involvement of signaling pathways related to cell proliferation, apoptosis resistance, and cell migration and invasion. This proteomic approach identified 27 known potential markers, including ANXA1, 14-3-3 Sigma, TGFB1, and POSTN. Five selected candidates were further evaluated in PDAC by immunohistochemistry analysis of tissue microarrays. Five interesting proteins, CD59, CD70, CD74, TGFBI, and TGM2, were further evaluated on tissue microarrays. CD74 was identified, for the first time, as highly upregulated in PDAC by using the proteomic approach, and immunohistochemical study with accumulating evidence indicating that it plays important roles in tumorigenesis and angiogenesis. These results not only demonstrate that this proteomic approach is a useful tool for marker discovery, but contribute to further understanding of pancreatic tumorigenesis and development of novel therapeutic targets to improve its treatment.
Modern biological and medicinal researchers increasingly rely on large-scale technologies for the comparative analysis of proteomes. To satisfy this growing demand, maturing proteomic methods must achieve high-throughput, quantitative analyses of near-complete proteomes. Here we describe two efforts in this vein.

The first is the identification of a comprehensive yeast proteome from just over an hour of analysis time. We achieved this expedited proteome characterization through improved sample preparation, chromatographic separations, and by using a new Orbitrap hybrid mass spectrometer equipped with a mass filter, a collision cell, a high-field Orbitrap analyzer, and, finally, a dual cell linear ion trap analyzer (Q-OT-qIT, Orbitrap Fusion). Over an 1.3-hour method, the Q-OT-qIT hybrid collected an average of 13,447 MS1 and 80,460 MS2 scans per run to produce 43,400 peptide spectral matches and 34,255 peptides with unique amino acid sequences. These experiments delivered an extraordinary 67 proteins per minute and demonstrate that complete analysis of the yeast proteome can be routinely performed.

The second is a quantitative approach that blends the accuracy of SILAC with the impressive throughput of isobaric tagging methods. The strategy – neutron encoding (NeuCode) – relies on the mass defect of atoms and their isotopes and harnesses the exceptional resolving power of modern FT-MS systems to selectively reveal or conceal quantitative information when desired. When compared to traditional SILAC, NeuCode SILAC achieves comparable quantitative accuracy and improved proteome coverage. When compared to TMT labeling, NeuCode SILAC's wider dynamic range substantially improved discovery potential. Finally, we combined NeuCode SILAC with reductive dimethylation to compare the salt-stressed yeast proteome in up to 18 different conditions. These results demonstrate that NeuCode SILAC can be configured to accommodate diverse proteomic experiments.
Native mass spectrometry is now routinely used to measure m/z of intact protein complexes. To gain substructure information on the complexes an activation method in a tandem mass spectrometer is needed. Ion Mobility is also used to provide information on collisional cross sections and unfolding pathways. In this presentation, the use of surface-induced dissociation (SID) coupled to ion mobility will be illustrated. Ion mobility can be used either before or after the surface collision and two steps of surface activation can be used as shown in the figure. Data for a number of protein-partner complexes will be presented, where the partner can be small molecule ligand, protein, DNA, or RNA. Fragmentation to subcomplexes will be shown and the influence of charge on the fragmentation will be illustrated. The results illustrate that substructure information is available through the surface-induced dissociation method. In addition, SID spectra reflect well any initial structural changes caused by “heating” within the instrument. SID-IM-SID will be illustrated and it will be shown that substructural information is provided, along with information on charge partitioning.
Polyethylene terephthalate (Pe; overhead transparencies) can be exploited as the substrate for microfluidic devices with printer toner (T) used as adhesive to generate rotation-driven microdevices (RDMs) for a variety of analytical processes. We demonstrate that CD-like PeT microchips can be fabricated for fluidic transport controlled by centrifugal forces and valving by laser-printed hydrophobic valves. By simply using rotation speeds that generate enough centrifugal force, fluids could be mobilized through open channels and, at higher speeds, through hydrophobic valves. In a PeT RDM with two fluidic layers fabricated by laser ablating the microfeatures in the Pe sheet and then laminating using the toner for bonding, we show that a simple colorimetric protein assay can be carried out in an automated manner. In doing so, we highlight the ability to integrate a substantial number of fluidic control elements to achieve a basic diagnostic functionality (i.e., protein quantification). The functionality requires fluid metering, mixing, and aliquoting, and thus, represents an effective demonstration of fundamental operations required for a broad range of other applications. One of these involves cell counting via DNA quantitation based on the extent of magnetic bead aggregation as the optical signal. A bi-directional rotating magnetic field (RMF) was critical to effective magnetic actuation of the PeT RDM for multiplexed cell counting. Enumeration of white blood cells in the human blood samples was demonstrated with the RDM with reasonable accuracy for clinical applications (CV=10%). Fusing the cost-effective and versatile PeT RDM with a simple bi-RMF, we present a promising strategy for automation and multiplexing of limited blood count chemistries.

Keywords: Analysis, Bioanalytical, Clinical Chemistry, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
In this talk I will focus on micro systems and automation tools to address questions in developmental neurobiology and immunology. We have developed a series of microfluidic systems that are fully automated to handle, image, and sort C. elegans, a nematode model system for many neuroscience studies. We combine this system with advanced computer vision and statistical techniques to identify and study genes involved in synapse formation. The advantages of this approach include not only the two orders of magnitude throughput improvement but also the ability to find mutants that were previously not possible to find. A second set of microfluidic systems that I will cover in this talk have applications in systems immunology. We take advantage of the ability to create and control sophisticated microenvironment that may vary in both time and space to study T cell signaling dynamics.
Lipid enzymes such as sphingosine kinase (SK) play critical roles in regulating the function and behavior of cells and their dysregulation is known to be an important factor in a variety of diseases. SK is a crucial regulator of cell proliferation, survival, and differentiation implicated in diseases involving the immune system, including leukemia and autoimmune disease, and a number of compounds are in use or in clinical trials aimed at SK as a molecular target. The power of capillary electrophoresis for single-cell applications will be shown by direct measurements of SK activity in cells of patients with leukemia and in primary natural killer cells of healthy subjects. An automated microelectrophoresis system facilitated rapid separation and quantification of the phosphorylated and nonphosphorylated SK reporter as well other metabolic products of the SK pathway. Single-cell analysis rates of 4 cells/min enabled data collection on hundreds of cells to achieve statistically significant results. Measurements in primary peripheral blood mononuclear cells from patients with different forms of leukemia demonstrated significant intercellular and inter-patient SK heterogeneity as well as in the amounts of other metabolites formed. The variability in SK signaling has important implications for SK inhibitors as therapeutics for leukemia and demonstrates the value of single-cell analysis in characterizing the nature of oncogenic signaling in cancer. Similarly primary NK cells displayed a wide range of SK activity consistent with the very large numbers of NK-cell subclasses thought to be present in humans. Per cell quantification of signaling molecules can also be used to determine the effects of genetic perturbations or pharmaceutical inhibition on a cell-by-cell basis revealing cellular heterogeneity otherwise masked by traditional bulk assays.

Keywords: Bioanalytical, Biomedical, Capillary Electrophoresis, Electrophoresis
Application Code: Biomedical
Methodology Code: Capillary Electrophoresis
Microfluidic Innovations to Advance Molecular Analysis of Disease Pathways

Microfluidic Systems for Measuring Dynamics of Islets of Langerhans

Peptides released from islets of Langerhans, such as insulin, glucagon, and islet amyloid polypeptide, help regulate blood glucose. In vivo, these peptide levels are known to oscillate with periods ranging from minutes to hours. These complex secretion patterns have been shown to have a positive effect on peripheral tissue function, and loss of these patterns are observed in people with diabetes and their relatives. Although these oscillatory signatures are of importance, the mechanisms underlying their development are still unknown.

We have developed several micro-analytical systems that can measure various aspects of the dynamics of islets of Langerhans. In this talk, a method we have developed to simultaneously measure multiple peptides secreted from islets of Langerhans with ~60 s temporal resolution will be shown. This system is being used to examine the oscillatory secretion of peptides from single and groups of islets and how they may interact to form in vivo oscillations. We will also describe a microfluidic device that can be used to mimic the in vivo interactions between the pancreas and peripheral tissues which can produce oscillations with similar time periods as those observed in vivo.

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Microfluidic Innovations to Advance Molecular Analysis of Disease Pathways

Integrated Microfluidic Molecular Analysis of Circulating Exosomes

Developing non-invasive blood-based tests is extremely appealing for presymptomatic screening and early detection of cancer where tissue biopsy is highly invasive and costly. Probing circulating exosomes is an emerging paradigm for cancer diagnosis and monitoring of treatment response. However, biology and clinical value of exosomes remain largely unknown, due in part to current technical challenges in rapid isolation, molecular classification and comprehensive analysis of exosomes. In this presentation, we will discuss on a microfluidic approach to streamline the exosome analysis pipeline via integrating immunoisolation and targeted proteomic analysis of exosomes. This method enables quantitative exosomal biomarker analysis directly from minimally invasive amount of plasma samples within ~100 min, with markedly improved detection sensitivity. We demonstrated phenotyping of exosome subpopulations by targeting common exosomal and tumor-specific markers and multiparameter analyses of intravesicular biomarkers in a selected subpopulation. We were able to assess the total expression and phosphorylation level of IGF-1R by probing circulating exosomes in non-small cell lung cancer patients, as a non-invasive alternative to conventional biopsy and immunohistochemistry. We foresee the microfluidic exosome analysis platform will form the basis of critically-needed infrastructure for advancing the biology and clinical utilization of exosomes.
Primary hyperparathyroidism, a condition which can be debilitating or even fatal, is caused by the overproduction of parathyroid hormone (PTH) by one or more parathyroid glands. The increased PTH stimulates bone resorption, causing deleterious, elevated calcium concentrations in the blood. The standard of care is to remove the overproducing parathyroid gland as identified, if possible, with medical imaging and then determine whether additional glands need to be removed during the surgical procedure. Fortunately, PTH has a very short circulating half-life, and the removal of additional glands is conducted if the systemic level of PTH does not drop to less than 50% of the original levels after 10 minutes. While in some cases, the FDA-approved systems for PTH quantification can be operated proximally to the surgical suite, this service requires a dedicated technologist and is generally not available nationwide. Typically the immunoassays for PTH are performed in a central laboratory. Primarily due to transport times, the desired information may take over an hour to be returned to the surgeon following the removal of the primary gland. These long turnaround times cause undesirable risks and costs to the parties involved. This work aims to decrease the actual time-to-result in the clinical setting by generating a point-of-care diagnostic capable of being implemented in the workflow of the operating room. Herein, we describe the unique design requirements to achieve faster turnaround times in the operating room and present progress to date in developing a simple, portable diagnostic for PTH utilizing a fluorescent sandwich immunoassay.

Keywords: Bioanalytical, Biomedical, Biosensors, Immunoassay
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Platforms for Point-of-Care Analysis

Towards Point-of-Care Analysis Using Digital Microfluidics

There is great enthusiasm in the microfluidics community for the development of “lab on a chip” systems for portable, point-of-care (POC) analysis. A major challenge in such applications is sample preparation—in particular, bridging the gap from the macro-scale of clinical specimens to the micro-scale of efficient fluidics/detection. The last decade has seen important advances in this area, but most systems today are either (a) very simple, with modest capability to integrate sophisticated sample processing protocols, or (b) require extensive off-chip ancillary equipment to operate (pumps, valves, tubes, interconnects, etc.). In this talk, I will present work towards an alternative scheme for POC analysis that relies on “digital microfluidics” (DMF). In DMF, droplets of sample and reagents are manipulated electrostatically on open devices (with no channels) bearing an array of electrodes covered with a hydrophobic insulator. DMF has the potential to address the challenges described above, allowing for sophisticated, multi-step sample processing on devices that operate with minimal external resources. I will highlight two different applications: on-chip immunoassays for diagnosing infectious diseases (including congenital rubella syndrome) and portable mass spectrometry-based quantitation of drugs of abuse for workplace testing. These examples and others suggest that digital microfluidics may be a useful new tool in the area of POC analysis.

Keywords: Biological Samples, Clinical Chemistry, Lab-on-a-Chip/Microfluidics, Mass Spectrometry

Application Code: Clinical/Toxicology
Methodology Code: Microfluidics/Lab-on-a-Chip
In 2010 the Medimate Multireader® was introduced by Floris et al. as the first electrophoresis based self-test for monitoring charged species, specifically to quantify lithium in finger-stick whole blood. Since its introduction, improvements have been realized in accuracy, precision, detection limit and reliability. This was achieved through improvements in temperature control, ease of use, connector stability, and the use of multiple injection and integrated data-analysis. Here the latest test results are presented for quantification of various species, most extensively for lithium for laboratory and home testing, but also creatinine, calcium and magnesium in whole blood and sodium in urine. By developing the method of repetitive measurement, using a single prefilled lab-chip cartridge, with alternating polarity, we furthermore became able to determine in our platform both positively and negatively charged species in one single run on one sample, a process we call cation and anion electrophoresis (CAE). Measurement results are presented for 7 different sample types. By using this method it positive and negative species can be measured in one analysis run, while it is anticipated that accuracy can be improved and that the dependency on an internal standard can be reduced. The improvements presented will bring the chip-based CE platform closer to point-of-care application.

Figure 1. Multireader CAE separation for serum. Right from the zero line a cation electropherogram, to be read from zero time left to right. Left from the zero line the anion electropherogram, to be read from right to left and with a resistance offset correction at zero time. Please note that these electropherograms are taken from one sample and one chip, by subsequent switching of the injection and separation voltages.
Infectious pathogens often cause serious economic loss and public health concerns throughout the world. One important characteristic of infectious diseases is that they often occur in high-poverty regions, where people cannot afford expensive and bulky equipment. Although numerous polydimethylsiloxane (PDMS) and paper-based microfluidic devices have been developed to address this issue, PDMS/paper hybrid systems that take advantage of both substrates are rarely reported. Each device substrate has its own advantages and disadvantages. Herein, we have developed different low-cost PDMS/paper hybrid microfluidic systems that take advantage of both PDMS and paper substrates for rapid and sensitive infectious disease diagnosis, especially in low-resource settings. For instance, the novel use of the paper substrate used in a hybrid microfluidic system facilitated the integration of graphene oxide nanosensors on the chip for one-step food-borne pathogen detection, and avoided complicated surface treatment for nanosensor immobilization in a PDMS or glass-only microfluidic system. Additionally, paper was used in another PDMS/paper hybrid microfluidic system integrated with loop-mediated DNA isothermal amplification (LAMP) for rapid and sensitive meningitis diagnosis, a global disease with high morbidity and mortality. The introduction of paper into the microfluidic device enables stable test results over a much longer period of time than a paper-free microfluidic system. Results can be observed by the naked eye. Although this hybrid system does not require expensive instruments, its sensitivity is even higher than conventional real-time PCR. Financial support from NIH, UT STARS Award, MRAP, IDR2 and URI award from UTEP is gratefully acknowledged.
LAMP is an isothermal amplification technique that uses the BST polymerase and 6-8 primer sets to perform sensitive and specific amplification of DNA or RNA targets via strand displacement and rolling circle amplification. A billion copies of a target can be generated in one hour. Quantitative detection can be accomplished by densitometry (since MgPO₄ is a precipitant product of amplification) or by adding a DNA adduct dye and exposing to light. The reaction is far more robust to variations in temperature, time, and inhibitors than PCR. The reaction has been used as an established diagnostic tool for infectious disease pathogens, including HIV and SARS. To make LAMP even more sensitive and robust for resource poor or point-of-care settings, we describe the implementation of digital LAMP (dLAMP) in a SD (Self Digitization) chip that we have developed over the past several years. In dLAMP, the sample is divided into an array of small volumes such that each volume contains only one copy of the DNA or RNA molecule in the sample while the majority of the volumes contain no DNA molecules. Isothermal amplification of the array of volumes result in fluorescence (or another signal) only in the few small volumes that each contains a single copy of DNA. The DNA copy number is easily and accurately determined by simply counting the number of small volumes that contain a copy (i.e. fluorescent). We describe the advances we made to translate this SD platform for use in the low-resource and point-of-care setting.
Degradation of paintings and other cultural heritage artifacts occurs through classical chemical reactions which depend on the chemical nature of materials and environmental conditions. However, the kinetics of these chemical reactions operates under extremely unusual conditions due to the condensed and structured materials in which they take place. This gives them an apparent chemical specificity which goes well beyond the much increased time constants. Such specific kinetic aspects and constraints are generally not taught in chemical courses though they are essential for preventing or restoring degradation.

This talk will focus on general issues concerned with the importance of these specific constraints based on classical chemical examples and, as much as possible, several illustrations taken from real artifacts deteriorations.
Recovering the Original Colors of Paintings through Analytical Chemistry

Synchrotron-Based Methods for Studying the Alteration Mechanisms of Cadmium Sulfide and Arsenic Sulfide Pigments in Early Modernist and Dutch Golden Age Paintings Respectively

Cadmium carbonate (CdCO₃) has been identified in the altered cadmium yellow (CdS) paints found Impressionist, early modernist, and post-Impressionist paintings. When it is concentrated at the surface of the paint layer, CdCO₃ appears to be a result of the photo-alteration of CdS. However, in other cases CdCO₃ is distributed throughout the paint layer. CdCO₃ is highly insoluble (Ksp of 1.0 ×10⁻¹²) and would not be expected to migrate from the painting’s surface. Plahter et al. have recently proposed that CdCO₃ is present in cadmium yellow paints as residue from the indirect wet process synthesis of CdS using CdCO₃ and Na₂S. To determine the role of CdCO₃, as well as cadmium oxalates and sulfates, microspectroscopy and microdiffraction were carried out at ESRF ID21 and Petra III. X-ray fluorescence mode (XRF scanning) allowed precise mapping of elemental distribution, and Full Field X-ray Near-Edge Absorption Structure (FF-XANES) allowed mapping the chemical speciation of cadmium and sulfur. FF-XANES CdCO₃ distributions support Plahter’s hypothesis for a 1906 work by Henri Matisse.

Yellow orpiment (As₂S₃) and red-orange realgar (As₄S₄) are arsenic sulfides that have been used as pigments since antiquity. Orpiment becomes paler and colourless upon exposure to light, while realgar turns bright yellow. The photo-oxidation process involves the conversion of orpiment to colorless arsenic trioxide (arsenolite, As₂O₃) and realgar is altered to pararealgar (As₄S₄, an isomer of realgar), and then eventually to arsenic trioxide. This paper will present the µXANES identification of further degradation products of realgar, and their subsequent mobility in different paint systems.

Abstract Text
Cadmium carbonate (CdCO₃) has been identified in the altered cadmium yellow (CdS) paints found Impressionist, early modernist, and post-Impressionist paintings. When it is concentrated at the surface of the paint layer, CdCO₃ appears to be a result of the photo-alteration of CdS. However, in other cases CdCO₃ is distributed throughout the paint layer. CdCO₃ is highly insoluble (Ksp of 1.0 ×10⁻¹²) and would not be expected to migrate from the painting’s surface. Plahter et al. have recently proposed that CdCO₃ is present in cadmium yellow paints as residue from the indirect wet process synthesis of CdS using CdCO₃ and Na₂S. To determine the role of CdCO₃, as well as cadmium oxalates and sulfates, microspectroscopy and microdiffraction were carried out at ESRF ID21 and Petra III. X-ray fluorescence mode (XRF scanning) allowed precise mapping of elemental distribution, and Full Field X-ray Near-Edge Absorption Structure (FF-XANES) allowed mapping the chemical speciation of cadmium and sulfur. FF-XANES CdCO₃ distributions support Plahter’s hypothesis for a 1906 work by Henri Matisse.

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Keywords: Art/Archaeology, Paint/Coatings, Speciation, X-ray Fluorescence
Application Code: Art/Archaeology
Methodology Code: X-ray Techniques
The study of Cultural Heritage materials requires advanced techniques to shed new lights on ancient technologies and help in their preservation. The implementation of new analytical tools, including mobile laboratories, allows a deep insight on the archaeological and artistic materials. We will show the performances of a new mobile XRF/XRD instrument we built to allow in situ characterization of the nature, the alteration and the mode of preparation of different pigments. The precious character of the most famous works of art and their uniqueness imply particular cautions and require instruments which may give the maximum of information directly on the artefacts, in-situ in the museums or in the archaeological sites.

Keywords: Art/Archaeology, Instrumentation, X-ray Diffraction, X-ray Fluorescence

Application Code: Art/Archaeology

Methodology Code: X-ray Techniques
### Abstract Text

In paintings of individuals, artists often make large compositional paint changes such as recycling the canvas for a new portrait or making changes in the head and face of a sitter. Examples include Blue Period works by Picasso such as “Le Gourmet” (National Gallery of Art), which depicts a young boy but is painted over a Spanish woman wearing a mantilla. In this talk we will show the utility of combining information obtained from hyperspectral imaging using three modalities, reflectance (400 to 2500 nm), luminescence (500 to 1000 nm), and x-ray fluorescence (2 to 15 KeV) spectroscopies, to help understand the spatial features and pigments used in the earlier compositions. Case studies will include several Blue Period Picasso paintings and Fragonard’s “Young Girl Reading.” Finally, the use of reflectance imaging spectroscopy to develop a more advanced model of the optical properties of aged varnish will be discussed. These types of model have been used in the virtual cleaning of an Old Master painting in which the varnish layer degraded over time, causing an alteration to the appearance of the painting.

**Keywords:** Art/Archaeology, Near Infrared, Vibrational Spectroscopy, X-ray Fluorescence

**Application Code:** Art/Archaeology

**Methodology Code:** Other
A group of five paintings on canvas known as Mark Rothko’s Harvard Murals (1962) has changed color due to the presence of a fugitive red pigment (Lithol Red) and excessive exposure to natural light in a room with large windows. This project explores the possibility of recreating the original color appearance by using a digital projector as illumination. A camera-projector system determines the characteristics of the painted surface and the projector’s non-linear response. By comparison with a target image we calculate a corrective image that is projected onto the paintings recreating the original color appearance.

Restored Kodak Ektachrome photographs of the paintings in their original state from 1963 serve as a reference. These transparencies which have also faded over the last 50 years are scanned using narrow bandwidth light sources ensuring that the recorded optical densities are proportional to the photographic dye concentrations. The original color of the Ektachromes is then digitally reconstructed using a chemical fading model based on these dye concentrations.

With a camera-projector system we calculate the geometric mapping and the radiometric compensation. The geometric mapping yields the coordinate transformations between the projector, the camera, and the digital target image. Subsequently, the color mixing matrix and nonlinear projector response are calculated. With all parameters of the system known, we can calculate the compensation image pixel by pixel and project it with the correct registration onto the faded canvas.

This novel restoration technique uses colored light from a digital projector to compensate for color alteration. It recreates the original color appearance without physically changing the paint surface and is therefore completely reversible. Microfading tests insure that the paintings will not continue to fade as a result of this treatment. As a result, Mark Rothko’s Harvard Murals can be viewed and experienced as in 1962.

Keywords: Art/Archaeology, Computers, Software
Application Code: Art/Archaeology
Methodology Code: Computers, Modeling and Simulation
Most interesting properties of mid-IR quantum cascade lasers are their high spectral power density, continuous wave or pulsed operation with hundreds of kHz, as well as the coherent and polarized nature of the emitted light. This presentation will report on novel analytical applications ranging from environmental and industrial process monitoring including gases (H2S, CS2, NO, NO2) and liquids (oil in water) to near field imaging. The latter application is based on photo expansion spectroscopy and employs pulsed QCLs and an atomic force microscope. Concerning each application the unique properties of the employed QCL are crucial for successful problem solving.
The development and performance of materials with specific desired properties, such as strength, ductility, durability, resistance to fracture, opacity, conductance, etc., often depend on sub-micrometer-sized structural features. Understanding the chemical nature of these small features can potentially lead to more rapid convergence on formulation parameters and the efficient production of superior materials. Although surface characterization techniques such as atomic force microscopy (AFM) provide high resolution imagery of the topography of sub-micrometer-sized features, little chemical information is typically derived from these images. Infrared (IR) spectroscopy, on the other hand, is a well-established chemical characterization technique capable of identifying specific molecular components, including their polymorphic form, molecular orientation, and specific chemical environment. Unfortunately, conventional Fourier transform infrared (FT-IR) microspectroscopy instrumentation, does not provide this information at high enough spatial resolution, because it is limited by diffraction physics to be on the order of the wavelength of light used to make the measurement (3-10 micrometers). The recent development of near-field mid-IR spectroscopic approaches, which detect the IR spectral information at the small-diameter tip of an AFM, have improved the spatial resolution by about two orders of magnitude. Several examples are presented which demonstrate how this powerful nanoscale chemical characterization capability can help provide important understanding of how material component interactions relate to the ultimate properties of the material. Examples include polymer blends and composites, microdomain-forming polymer films, and oriented fibers.

Keywords: Imaging, Infrared and Raman, Materials Characterization, Microscopy
Application Code: Materials Science
Methodology Code: Molecular Spectroscopy
The use of label-free methods for imaging is rapidly emerging as an alternative to conventional labeling techniques. In particular, the use of spectroscopy to image molecular content is termed chemical imaging. We present first progress based on mid-infrared chemical imaging that combines the spatial specificity of optical microscopy with the molecular selectivity of vibrational absorption spectroscopy. Instead of directly imaging probes, recorded data are related to the structural and functional state of the biological material using computation. This process is actually not straightforward and requires a deep understanding of the underlying optical physics and light-matter interaction. An overview of the recently developed theoretical approaches is provided. A combination of theory, re-designed instrumentation and signal processing forms an integrated approach to biochemical analyses, especially for histopathology. Development of this technology will enable the rapid analysis of cells and tissue by fingerprinting the inherent biologic content, extraneous materials and metabolic state without the use of probes. In a variety of applications, we first describe attempts to diagnose and grade cancer in breast and prostate biopsies without human input. Results indicate that a rapid assessment of lesions is possible with high accuracy and their lethality may be predicted using a systems approach to pathology. Applied to engineered 3D tissue models for breast tumors, we show that the imaging technology is useful in rapidly assessing culture quality and that the model systems can act to inform researchers about the involvement of different cell types in cancer progression. Finally, we provide an overview of potential future directions and applications that will emerge from these studies.

Keywords: Biomedical, FTIR, Imaging, Instrumentation
Application Code: Biomedical
Methodology Code: Vibrational Spectroscopy
SPECIAL SESSION: International Year of Light (SAS)

Two-Dimensional Correlation Spectroscopy Study of Bioplastics

The critical role that two-dimensional (2D) correlation spectroscopy had played in designing and characterizing novel bio-based biodegradable plastics is described. In 2D correlation spectroscopy, a set of spectra defined by two independent variables, e.g., IR wavenumbers, is generated by applying a correlation analysis to spectral intensity changes induced by an external perturbation, such as mechanical deformation and change in temperature. Poly(3-hydroxyalkanoates) or PHAs are an interesting class of aliphatic polyesters found as granular intracellular inclusion bodies accumulated for energy storage by various microorganisms. Certain types of PHAs had been known for many decades, although their properties were rather limited to become a practical replacement for commodity plastics. Fortunately, 2D IR analysis of PHAs under mechanical perturbation provided a critical insight into the effective modification their molecular structure to overcome such shortcomings. More specifically, correlation peaks observed in 2D IR spectra indicated the surprising change in the local mobility of polymer chains even with a minor extension of some of the side groups of PHA. Although the improvement of the macroscopic properties at that level was still disappointingly modest, the result clearly suggested that further extension of side groups should result in achieving the dramatic improvement of properties. This discovery has led to the design and creation of a promising new class of PHA copolymers with excellent toughness and ductility, as well as a convenient range of thermal property for processing. The potential commercial utility of the newly developed bioplastics stimulated extensive studies including further 2D correlation spectroscopy analysis of such materials. Some of the interesting results obtained by the 2D spectroscopy study, such as sequential growth of different crystal structures, are presented.

Keywords: Infrared and Raman, Materials Science, Polymers & Plastics, Vibrational Spectroscopy

Application Code: Polymers and Plastics
Methodology Code: Molecular Spectroscopy
During the last few years, there has been an explosion of interest and activity in the field of plasmonics. The goal of plasmonics is to control and manipulate light on the nanometer length scale using the properties of the collective electronic excitations in noble metal films or nanoparticles, known as surface plasmons. An improved understanding of the interactions between adsorbed molecules and plasmonic nanostructures (i.e., molecular plasmonics) is having a significant impact in a number of research areas including electrochemistry, surface science, catalysis for energy conversion and storage, the materials science of nanoparticles, biomedical diagnostics, art conservation science, and nanolithography.

I will focus in on three recent advances in tip-enhanced Raman spectroscopy (TERS) which illustrate the power of this nanoscale vibrational spectroscopy. First, new insights into the nature of the relative intensity fluctuations in single molecule tip-enhanced Raman spectroscopy (SMTERS) will be discussed. Second, our current understanding of the adsorbate surface interactions involved in the low temperature (LT), ultrahigh vacuum (UHV) TERS of the Ag tip/Rhodamine 6G (R6G) /Ag(111) system will be described. Finally, an update on our new results in coupling ultrafast lasers with TERS. This last topic illuminates a path forward toward the goal of understanding chemistry at the space-time limit.

**Keywords:** Nanotechnology, Surface Enhanced Raman

**Application Code:** Nanotechnology

**Methodology Code:** Vibrational Spectroscopy
Terahertz (THz) technology has recently emerged as a potential imaging modality for several applications. THz imaging can achieve a higher resolution than microwave frequencies while also providing greater penetration than optical techniques. Current THz sources are low-power and non-ionizing, making them safe for tissue characterization in biomedical, security and industrial applications.

The differentiation of cancerous tissue compared to normal tissue is of particular interest. Furthermore, unique characterization of the signals reflected from and transmitted through the tumor will allow for rapid differentiation of cancerous and normal tissue regions prior to clinical sectioning. This research aims to develop a THz reflection imaging methodology for in-depth scanning of three-dimensional tissue blocks through comparison with THz imaging of tissue sections and standard pathology assessment. Frozen tissue obtained using snap freezing liquid Nitrogen and formalin-fixed and paraffin-embedded (FFPE) of breast cancer tissue will be demonstrated in this work.

For the security application of THz technology, it is known that dangerous and illegal substances and objects can be concealed in ways that make them difficult to detect using traditional methods (metal detectors, millimeter wave scanners). Examples of these materials are narcotics, powder explosives, ceramic knives, or plastic Guns. For industrial applications, our goal is to investigate the nondestructive inspection capabilities of THz imaging for quality control of power electronic devices and to protect proprietary x-ray blocking scattering (XBS) coating material. We established collaborative work with two local hi-tech startup companies in Arkansas; the Arkansas Power Electronics International and the Space Photonics Inc.

Keywords: Biomedical, Spectrometer
Application Code: Biomedical
Methodology Code: Other
Terahertz Spectroscopy and Imaging: Toward Practical Applications

Recent Practical Industrial Applications Using Terahertz Technology

Since the ultra-fast terahertz (THz) pulse radiation and detection has demonstrated in the late 80’s, many kinds of spectroscopy and imaging techniques have developed and are still progressing. In the past a few decades, many researchers have proved that THz spectral region is very attractive to industrial applications as well as fundamental science. In this symposium, we will present recent progresses of THz instruments and some industrial applications. We would say that significant technological breakthroughs in instrumentation that allow us to apply to industrial applications are miniature fiber coupled THz emitter/ detector and rapid THz wave sampling technology. The miniature fiber coupled THz emitter and detector give a flexibility and accessibility in measuring (or monitoring) the target materials flowing through production process. And the rapid THz wave sampling such as electronically controlled optical sampling technology opens the way to real time monitoring and high throughput testing. In recent years, for the sake of introducing of novel materials and production efficiency improvement, it is increasing the demand for new non-destructive testing solutions in various industries. THz technology is considered as one of the promising candidates for measuring thickness or material density of opaque materials. We will show some practical applications for pharmaceutical, semiconductor and other industries.

Keywords: Pharmaceutical, Polymers & Plastics, Process Monitoring, Spectroscopy
Application Code: Quality/QA/QC
Methodology Code: Other
Recent practical advancements in THz source, detector, and system technology have enabled researchers to explore a myriad of medical applications in both research and research laboratory and clinical settings. Due to the presence of water in physiological tissue the high THz absorption of water, and the relatively low absorption of non-water tissue constituents, reflective THz imaging is capable of producing high sensitivity surface tissue water content (TWC) maps with greater signal to clutter ratios than traditional medical imaging modalities. Variations in dielectric function due to TWC gradients have been measured in different tissue types and between cancerous and healthy tissues. These advantages coupled with the low, non-ionizing THz photon energy (0.4 - 40 meV) make THz imaging an ideal tool for in vivo imaging of skin burns, skin diseases, and corneal pathologies.

In this talk, a brief overview of the field is provided along with promising and emerging applications and ongoing research. THz electromagnetic models of tissue are presented that utilize Debye theory, effective media theory, and stratified media theory. These models are used to explore contrast mechanisms and predict imaging system performance. THz imaging system architectures are then discussed and tradeoffs are identified. A THz medical imaging system, operating at ~525 GHz center frequency with ~ 125 GHz of response normalized bandwidth is introduced and details regarding principles of operation are provided. Two promising medical applications of THz imaging are presented: skin burns and cornea. For burns, images of 2nd and 3rd degree burns were obtained in rat models in vivo over an 8 hour period. These images clearly show the formation and progression of edema in and around the burn wound area. For cornea, experimental data measuring the TWC of in vivo rabbit cornea are presented demonstrating utility in ophthalmologic applications.

Keywords: Bioanalytical, Biomedical, Biosensors, Imaging
Application Code: Biomedical
Methodology Code: Other
We introduce a new method for determination of drug binding using a previously underutilized terahertz frequency region. This frequency region corresponds to long range structural vibrations in proteins, which necessarily change with small molecule binding. Here we show dramatic changes in the vibrational spectra measured for the benchmark enzyme lysozyme and discuss applications to anti-tumor and anti-bacterial targets.

Keywords: Biospectroscopy, Drug Discovery, Molecular Spectroscopy, Protein

Application Code: Bioanalytical

Methodology Code: Vibrational Spectroscopy
Microdialysis is a sampling technique used to recover small molecules from the extracellular space of the brain for real-time chemical monitoring. Microdialysis has specifically been used to monitor the important neurotransmitter dopamine, and its involvement in drug addiction, behavior, and disease. However, inserting microdialysis probes into the brain damages the surrounding tissue, causing analytes to be collected from abnormal tissue. The extent to which this damage affects microdialysis recovery of dopamine is still completely understood. Coupling fast scan cyclic voltammetry with carbon fiber microelectrodes implanted near probes provides an in vivo calibration method to examine dopamine concentrations in the surrounding tissue. Previously we have found that inserting a probes into rat striatum diminishes stimulated dopamine responses at nearby microelectrodes. Stimulated responses can be restored by giving the dopamine uptake inhibitor nomifensine, proving that dopamine terminals survive near microdialysis probes. Dexamethasone, an anti-inflammatory steroid improves tissue health around microdialysis probes, by reducing the immune response to implantation, and protecting against ischemia and neuronal loss. Retrodialysis of dexamethasone also enhanced dopamine detection in brain tissue near microdialysis probes for 4 and 24 hours. Other drugs that target the immune response triggered by probe implantation may be useful in improving dopamine detection near probes in the brain, and further aid in our understanding of how probe induced tissue responses effect microdialysis recovery. This work was funded by the NIH (NS 081744).
Emerging Methods for Monitoring Neurochemicals in Living Systems

The Impact of Dexamethasone on Penetration Trauma Associated with Microdialysis Implants at 24 Hours

First introduced in 1966, the technique of microdialysis has been used for over four decades. Microdialysis has gained wide spread use for sampling and quantifying neurotransmitters in the brain. However, microdialysis has several disadvantages; it has poor temporal and spatial resolution and is considered an invasive technique. Profound consequences arise due to the impact of penetration trauma associated with the microdialysis probe; this is especially true with in vivo neurochemical measurements of dopamine (DA).

A prior study from our laboratory demonstrated the beneficial effects of retrodialysis of dexamethasone (DEX), a potent anti-inflammatory steroid, on the tissue penetration injury and DA concentrations in the tissue surrounding the probe during acute (4hrs) microdialysis experiments. Presently, we extended these findings to microdialysis experiments lasting 24 hrs. We used immunohistochemistry and fluorescence microscopy to compare, DA axons and terminals (TH) and dopamine transporters (DAT) near probes perfused with aCSF and DEX for 24 hrs. There were no significant differences in the TH and DAT labeling near the probe track. Since DA terminals in the striatum express both TH and DAT, the impact of microdialysis probes on their colocalization was determined using both Pearson’s Correlation Coefficient and Manders’ Overlay Coefficient. Overall, the probes had little effect on the correlation of TH and DAT labeling in the nearby tissue. We report here for the first time, that DEX serves to protect and stabilize the activity of the DA terminals near the probes, rather than increasing the number of surviving terminals during probe implantation at 24 hrs.

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Keywords: Electrochemistry, Microscopy, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Microscopy
As interest in developing [i]Drosophila melanogaster[/i] as a model for human neurological diseases continues to grow, characterization of the neurotransmitter content of flies becomes increasingly important. In addition to dopamine and serotonin, [i]D. melanogaster[/i] has invertebrate specific neurotransmitters, tyramine and octopamine, which fulfill similar roles to epinephrine and norepinephrine. In order to better understand the differences in tissue content of neurotransmitters between different developmental stages and different strains of flies, fast and sensitive methods for separation and detection of neurotransmitters in brain samples are necessary.

In order to quantify the neurotransmitter content of [i]D. melanogaster[/i] brains, our lab has developed capillary electrophoresis coupled to fast scan cyclic voltammetry (CE-FSCV). Using Canton S flies, tissue content of four neurotransmitters were determined across larva, pupa, and adult life stages. Separations of tyramine, serotonin, octopamine, and dopamine were achieved in under 10 minutes, and limits of detection were 9 ± 1 nM for tyramine, 3.0 ± 0.5 nM for serotonin, 12 ± 8 nM for octopamine, and 3 ± 1 nM for dopamine (n=4). Differences in tissue content between the different life stages were most obvious in the pupa, where tyramine levels were highest and octopamine levels were lowest compared to both larva and adults. In addition to developmental differences, we compare the adult tissue content of Canton S flies to w[sup]1118[/sup] flies. This comparison is important, as we have observed differences in tissue content of octopamine between wild type strains Canton S and Oregon R. Dopamine levels in w[sup]1118[/sup] flies are reduced relative to wild type flies and w[sup]1118[/sup] flies also differ to a smaller extent in their tissue content of serotonin.

Funding generously provided by the NIH (R01MH085159).

**Keywords:** Bioanalytical, Biological Samples, Capillary Electrophoresis, Electrochemistry

**Application Code:** Bioanalytical

**Methodology Code:** Capillary Electrophoresis
Emerging Methods for Monitoring Neurochemicals in Living Systems

Novel Glucose Oxidase-Modified Carbon-Fiber Microelectrode Biosensors Simultaneously Detecting Subsecond Glucose and Dopamine Fluctuations Utilizing Fast Scan Cyclic Voltammetry

Glucose provides the major energy source to the brain. As such, real-time molecular detection of glucose dynamics is imperative to understanding the regulation of brain energy utilization and its involvement in neuropathological disorders such as diabetes, as well as the molecular adaptations that occur upon exposure to substances of abuse. However, to date, existing methods for detecting brain glucose are limited in terms of temporal and spatial resolution. We have addressed this need by coupling our novel glucose oxidase-modified carbon-fiber microelectrode biosensors with fast scan cyclic voltammetry. With this approach, we can detect subsecond fluctuations of glucose with unprecedented selectivity and sensitivity, as well as high temporal and spatial resolution. Optimization of the voltammetric waveform has enabled simultaneous detection of glucose and dopamine at a single recording site. Future work will utilize these microbiosensors to provide insight into the molecular adaptations that occur in diabetes and after exposure to drugs of abuse. We acknowledge the National Science Foundation for their generous funding contributions on this project.

Keywords: Chemically Modified Electrodes, Electrochemistry, Microelectrode, Voltammetry

Application Code: Neurochemistry

Methodology Code: Electrochemistry
Emerging Methods for Monitoring Neurochemicals in Living Systems

Serotonin-Histamine Modulation in Parkinson’s Using Fast Scan Cyclic Voltammetry (FSCV)

Parkinson’s disease (PD) is a neurodegenerative disease that affects 1 million people in the United States alone. Despite wide research into the relief of the motor symptoms of PD, its non-motor symptoms like depression are largely unstudied. This is mainly due to the poor understanding of the effects of PD on neuromodulators other than dopamine. Histamine and serotonin are two important neuromodulators in PD, linked to many of its non-motor symptoms. To more clearly understand the actions of serotonin and histamine in PD, it is first crucial to understand how they modulate each other in different brain regions. In this work, we do this with fast scan cyclic voltammetry (FSCV) by simultaneously measuring serotonin and histamine with a single carbon fiber microelectrode (CFM). These measurements, combined with pharmacological manipulations enable us to perform a mathematical analysis on the extent, direction and magnitude serotonin/histamine modulation. We plan on taking our studies further and studying serotonin/histamine modulation in PD mouse models.

Abstract Text

Drugs, Electrochemistry, Electrodes, Neurochemistry

Bioanalytical

Electrochemistry
Trace level peptide measurements are important for biomarker and biological investigations. Such studies can be performed using microdialysis with immunoassay detection. However, immunoassays often lack sequence specificity and may require large sample volumes (>25 [micro]l). Here we describe advances in capillary liquid chromatography and multi-stage mass spectrometry (cLC-MS\sup n[/sup]) for measurements of attomole quantities of neuropeptides from low volume microdialysis samples.

To generate robust analyses, we utilized independent high and low pressure LC systems and two 6-port valves to engage loading/desalting and elution on a single 50 [micro]m i.d. reverse-phase column. The LC system is interfaced via a detachable emitter tip to a linear ion trap mass spectrometer (Thermo LTQ-XL). Initial results demonstrated high limits of detection (LOD) due to sample loss to tubing. To overcome this loss we optimized the addition of acetonitrile to samples and achieved sub-pM LOD for 5[micro]L samples (<5 amol) for multiple neuropeptides.

We applied this cLC-MS\sup n[/sup] technique to measure neuropeptide release following selective activation of specific neuronal populations. We investigated hypothalamic neuronal circuitry with DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) and observed dynamic release of neurotensin and orexin A. We investigated nucleus accumbens (NAc) circuitry using optogenetics and measured concurrent NAc dynorphin release. These findings validate this cLC-MS\sup n[/sup] technique for \[i\]in vivo[/i] neuropeptide studies and represent the first measurements of dynamic neuropeptide release with selective neuronal activation methods.

This work funded by the National Institutes of Health.

Keywords: Capillary LC, Liquid Chromatography/Mass Spectroscopy, Neurochemistry, Peptides

Application Code: Neurochemistry

Methodology Code: Liquid Chromatography/Mass Spectrometry
Emerging Methods for Monitoring Neurochemicals in Living Systems

Microfluidic Sensor System for Neurochemical Monitoring of Traumatic Brain Injury Patients

Spreading depolarization (SD), a spontaneous phenomenon linked to the development of secondary brain injury, is characterized by transient increase of extracellular potassium ($K^{+}$). In order to recover, the excess $K^{+}$ needs to be cleared and the ionic balance restored. This process is extremely energy-demanding. Repeated episodes of SD in the human injured brains have been shown to create a net energy deficit, the neighboring tissue becoming non-viable, hence resulting in secondary brain damage [1,2].

We are interested in the real-time neurochemical quantification of SD in the injured human brains using online microdialysis. To handle the small sample volume from clinical microdialysis, we have developed a microfluidic flow cell capable of resolving dynamic metabolic and chemical changes during these depolarization events [3]. The flow cell is fabricated on poly(dimethylsiloxane) (PDMS) by soft lithography and consists of an analysis chamber housing the biosensors and $K^{+}$ ion-selective electrodes. The system is used to monitor patients in surgery and in the intensive care unit. Lastly, a required feature for clinical instrumentation is self-calibration – an automated calibration system is also developed to track the performance of the sensors over time.


Keywords: Electrochemistry, Lab-on-a-Chip/Microfluidics, Microelectrode, Neurochemistry

Application Code: Neurochemistry

Methodology Code: Microfluidics/Lab-on-a-Chip
Abstract Text

Microdialysis is a powerful separation technique capable of monitoring the concentration changes of multiple analytes in the extracellular fluid of the brain. This technique generates small sample volumes in a continuous flow stream. Traditional (LC) methods used for sample analysis forfeit temporal information regarding dynamic processes due to the larger volumes necessary for analysis. Additionally, sample acquisition methods traditionally involve some form of tethering or anesthetizing the animal under study, greatly reducing the available behavioral information. In order to preserve both temporal resolution and behavioral information, the ideal analysis system is one that can be employed on-line, has fast analysis times of small sample volumes, and can be placed on a freely-roaming animal. In this study, we present an approach for coupling microdialysis sampling to microchip electrophoresis and electrochemical detection at a carbon electrode for monitoring neurochemicals in the dopamine metabolic pathway. The device is comprised of a double T PDMS/glass hybrid microchip with detection at two pyrolyzed photoresist film carbon electrodes in series, and was used to separate five analytes in the dopamine metabolic pathway in under 100 s. The developed method was used to monitor the dopamine metabolic pathway in vivo in both rat and sheep after the administration of L-DOPA. The complete device and associated instrumentation can be used remotely and on-animal, for near-real time in vivo monitoring.

Keywords: Bioanalytical, Electrochemistry, Lab-on-a-Chip/Microfluidics, Neurochemistry

Application Code: Neurochemistry

Methodology Code: Microfluidics/Lab-on-a-Chip
Surfaces and interfaces may exhibit spatial heterogeneity for many reasons, including chemical or structural inhomogeneity, or topographical effects. We have developed an approach to obtain spatial maps of these heterogeneities based on the premise that “probe” molecules exhibit varied interfacial dynamic behavior that is distinctively related to the local chemistry and topology. For example, the characteristic adsorption rate, surface residence time, and local molecular mobility (i.e. surface diffusion coefficient) are often exquisitely sensitive to the details of the local surface environment.

By choosing an appropriate probe molecule (or more than one molecule) and accumulating large numbers of molecular trajectories (generally several million are analyzed), the dynamic molecular behavior can be spatially partitioned and used to prepare spatial maps. The contrast mechanisms of these maps consist of absolute dynamic properties that can be directly compared to homogeneous surfaces that are prepared as calibration samples. A statistical multi-dimensional analysis then allows us to associate the local chemistry of a mixed surface to an appropriate calibration surface chemistry or mixture.

Having identified chemically- or physically-distinct surface regions, the behavior of molecules of interest can be partitioned by region. For example, the surface residence time of proteins can be measured simultaneously on crystalline and amorphous regions of polymer surfaces. Also, anomalously strong binding sites can be identified on filtration membrane materials or chromatographic media, and the influence of these sites on fouling and/or separation efficiency can be determined.

Keywords: Adsorption, Biotechnology, Chromatography, Microscopy
Application Code: Materials Science
Methodology Code: Microscopy
The development of surface coatings for advanced materials technologies usually requires that a large number of samples be prepared and individually tested to find the few that are best suited for a particular application. An alternative to this often-tedious process is to produce a chemical gradient in which the material composition varies along a single sample. Ideally, each gradient would exhibit gradual, monotonic compositional and properties variations extending from the macroscale down to molecule levels. In reality, they are usually much more complex. Unexpected properties variations appear on a wide range of length scales due to, for example, the spontaneous separation of the gradient components during film preparation. We seek to better understand the nanoscale-to-macroscale chemical and physical complexities of thin-film gradients prepared from organosilane precursors. These gradients are being prepared by both dip-coating and vapor-phase deposition methods. They have potential applications as new materials for chemical catalysis, chemical separations, tribology and tissue engineering. Optical single molecule (super-resolution) detection, spectroscopy and tracking are employed in their characterization. Single molecule tracking studies performed by wide-field video microscopy are used to assess the extent to which probe molecules adsorb to the film surface and to quantify the rates at which they diffuse through the films and across their surfaces under different ambient conditions, as a function of position along the gradient. Spectroscopic single molecule tracking methods are also implemented with the solvatochromic dye Nile Red to determine the local dielectric constant of the films.
Membrane transport plays vital roles in cellular functions. To better understand real-time transformation of cellular membrane of live cells and their molecular mechanisms, we have developed far-field photostable-optical-nanoscopy (PHOTON) and used it to characterize the dependence of membrane transport dynamics upon the size, charge and chemical properties of substrates (nanoparticles) in single live cells in real-time at single-molecule (SM) and nanometer (nm) resolutions. PHOTON includes photostable single nanoparticle imaging probes, single nanoparticle plasmonic microscopy and spectroscopy. The updated results and applications will be presented. The work is supported in part by NSF (CBET 0507036) and NIH (R01 GM0764401; 3R01 GM076440-04S1).
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**Abstract Text**

Single molecule methods for optical microscopy developed over the last two decades have allowed us to acquire an unprecedented level of detail about biological and synthetic nanoscale materials. A single experiment can generate gigabytes of high-noise, low information-density data about hidden heterogeneous processes. These realities make data analysis and interpretation a challenge. I will present some recent advances in processing and understanding single molecule dynamics at interfaces that make use of information theoretic principles and that can be applied to a broad range of biological and synthetic nanomaterials.

**Keywords:** Fluorescence, Imaging, Nanotechnology, Separation Sciences

**Application Code:** Bioanalytical

**Methodology Code:** Fluorescence/Luminescence
Receptors are key components of the cell membrane for coordinating the passage of signals into and out of cells. Diffusion is an important mechanism for this function. We are elucidating the role of diffusion in the function of two receptors: integrins and receptor for advanced glycation end products using fluorescence recovery after photobleaching (FRAP), single particle tracking (SPT) with ligand-labeled quantum dots, and stimulated emission depletion imaging (STED). The role of post-translational modification and ligand structure in altering receptor diffusion is discussed. Among our interesting findings, the covalent attachment of palmitic acid on cysteine residues decreases the mobility of alphaPS2CbetaPS integrins as measured by FRAP and SPT. Additionally, the size of domains of confined diffusion increases after blocking a reported palmitoylation site. STED imaging is a subdiffraction technique that enables the organization of the cell membrane to be measured with 40-nm spatial resolution. The correlation between the cell membrane composition and the actin cystoskeleton is measured using STED imaging. Our work provides vital information on the molecular mechanism of receptor function through altered dynamics and membrane organization. This work is funded by the National Science Foundation (CHE-0845236 and CHE-1412084).
We have been exploring the electric fields that arise when nanoparticles interact with other metal surfaces and nanoparticles. The resulting electric fields enable chemical sensitive detection, an effect commonly known as surface enhanced Raman scattering (SERS). Here we will discuss the direct determination of electric fields using nitriles as vibrational Stark probes. Using tip-enhanced Raman scattering (TERS) we can control the interaction between structures and enable high-sensitivity chemical analysis. For example, the resulting electric fields have been shown to enable chemical selective studies on cellular membranes. We have selectively detected integrin $\alpha_3$ in the membrane of a cultured cell by binding an RGD-functionalized nanoparticle to the integrin and detecting the interaction via TERS. This approach appears to overcome challenges common to TERS imaging, specifically locating the protein on the vast membrane landscape and discriminating the protein of interest from the surrounding biomolecules. Fundamental studies of the electric fields around plasmonic particles are enabling new possibilities in chemical analysis.
Optical Imaging: On Liquid-Solid Interfaces and Cell Membranes

Reversed-phase liquid chromatography is a widely used technique for separation of analytes in both research and applied settings. The stationary phase material usually consists of micron-sized, mesoporous silica particles functionalized with n-alkane ligands. Understanding the timescales at which analyte molecules are transported through the interior of the particle as well as adsorbed and desorbed from the particle surface is of fundamental importance in the development of more efficient chromatographic materials. The majority of the surface area in these mesoporous silica materials is contained within the particle, making it difficult to observe these processes directly. Intra-particle transport kinetics have generally been inferred from chromatographic retention data. Spectroscopic techniques can provide evidence of dynamics at reversed-phase chromatographic interfaces, but these have generally been conducted at model, planar silica surfaces. In this talk, imaging-fluorescence correlation spectroscopy is used to probe transport of the amphiphilic fluorescent probe molecule, 1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate (DiI), within actual RPLC porous silica particles. Measured parameters are compared with those determined on C18-modified glass coverslips used as planar analogs of RPLC surfaces. Molecular transport within the particles is dominated by surface diffusion due to the high retention conditions. Surface diffusion appears slower within the particle as molecules diffuse along the contours of the interior surface of the particles. The 3-dimensional intraparticle surface diffusion, however, is projected onto a 2-dimensional image, thus the measured transport parameters are not directly comparable to those measured on planar model surfaces. We present an analysis to compensate for the change in dimensionality between porous particles and planar surface to facilitate the comparison of measured parameters in both systems.

Keywords: Fluorescence, Microspectroscopy, Modified Silica, Raman
Application Code: Materials Science
Methodology Code: Microscopy
We track three-dimensional (3D) trajectories of negatively charged nanoparticles being retained near negatively charged glass surface using astigmatism-based 3D super-localization fluorescence microscopy. We show that retention of nanoparticles by the surface can happen at the long distance without significant contribution from adsorption. That is, these particles can be trapped in potential energy minima several tens to hundreds of nanometers from the surface by long-range interactions between the particle-surface attractive site(s) and particle-surface. Trapping center, both attractive and repulsive. The trapped particle diffuses actively in the potential energy well with a lateral size on the order of magnitude of 1 micrometer squared before being immobilized on surface or diffusing away. This view may shed new light on how biomolecules/nanoparticles interact with a solid or semi-fluidic membrane surface. All data is analyzed by NIH ImageJ and Matlab.

This work is supported by NCSU startup to G.W.

Keywords: Fluorescence, Liquid Chromatography/Mass Spectroscopy, Method Development, Microscopy
Application Code: Bioanalytical
Methodology Code: Microscopy
Preeclampsia (PE), a pregnancy-related disorder, is characterized by hypertension and proteinuria. Currently, PE cannot be predicted. There would be value in having serum biomarkers able, early in a pregnancy, to identify women at substantial risk for PE. Known PE biochemical abnormalities likely alter the lipid profile of individuals at risk of PE. Therefore, a novel, serum lipidomic analysis of sera from early gestationally-aged pregnant women was undertaken to find useful predictive PE biomarkers. Sera obtained at 12-14 weeks pregnancy from 29 asymptomatic PE cases and 28 controls were processed by hexane/isopropanol extraction (3:2). Direct infusion of organic phase to electrospray-ionization-time-of-flight mass spectrometry (ESI-TOF-MS) was performed followed by analysis of MS data. Peaks having statistically significant quantitative differences (p<0.05) were considered candidate biomarkers. Fragmentation studies to characterize candidate markers using collisionally-induced dissociation are underway. To substantiate utility of candidates, a blinded set of 46 PE cases and 46 controls have been processed and submitted to ESI-TOF-MS. Analysis has uncovered 60 statistically significant serum lipid markers comparing control sera (uncomplicated pregnancies) to sera from cases who developed PE 3-5 months later. The most statistically different serum lipid biomarkers with m/z and p-values are listed in Table 1. Some lipids have been tentatively classified based on LipidMaps (Table 2). Studies to identify the rest of the candidates and evaluate the detected markers blindly are underway. This serum lipidomic approach found several promising predictive serum markers for later PE in pregnant women at 12-14 weeks gestation.

The funding has been provided by a Technology Bridging Grant from BYU and a CRISP grant from the College of Physical and Mathematical Sciences at BYU, Provo, UT

Keywords: Lipids
Application Code: Biomedical
Methodology Code: Mass Spectrometry
A heart attack can occur when coronary arteries become blocked due to coronary artery disease (CAD). After a heart attack, scar tissue forms in the damaged muscle. The remaining heart muscle works harder to pump blood through the body. In humans this "scar" tissue remains and can not be repaired. In contrast the Zebra Fish has the ability to regenerate the effected heart muscle and remove the scar. An complete understanding of the biology process may lead to a new approach to treatments for human in the future. To investigate the biochemistry behind this repair in fish three groups of Zebra fish (heart operation, sham operation and controls) were analysed by proteomics, metabolomics and lipidomics methodology. Due to the low sample volume an common platform micro-LC accurate mass MS/MS approach was employed for all of the analysis. The resulting data was processing separately via a multivariate statistical approach using PCA, OPLSDA etc. The proteomics data was subjected to a multi data base search to identify proteins involved in up-regulated pathways. The resulting data showed clear differentiation between the three groups of samples. With the metabolism and protein data being combined to identify upregulated biochemical pathways specific to fish biochemistry.
Biomedical Applications for Mass Spectrometry

Investigating the Mechanism of Preeclampsia by Probing the Low Molecular Weight (LMW) Placental Proteome Using Capillary Liquid Chromatography-Orthogonal Time-of-Flight Mass Spectrometer (cLC/Q-TOF-MS)

Preeclampsia (PE) is a potentially fatal complication of pregnancy whose cause is poorly understood. There are no disease-changing therapies except delivering the placenta. There are several proposed theories for PE but none has been proved. The aim here is to expose normal human placental explants to conditions proposed to be causal in PE, and study changes in the LMW proteome for each condition using cLC/QToF. The results will be compared to PE placenta analyzed by the same proteomic approach to determine which alterations are found in actual PE placenta, thus confirming involved pathway(s). To simulate PE conditions, in vitro, placenta were collected (n=12) from uncomplicated, elective C-sections. Each explant was exposed to a hypothesized PE condition. Control explants from the same placenta were cultured for the same time, 48 hr, in a normoxic environment. Each explant was homogenized and acetonitrile precipitation was performed to remove abundant, high molecular weight proteins. A defined amount of protein-depleted sample was injected onto the cLC/MS for analysis. MS spectra for each treatment and controls were compared visually and potential differences evaluated statistically. Data analysis is still underway but 62 markers having significant differences between placental explants incubated under normoxic versus hypoxic conditions have been found. One of these markers was identified as a fragment of hCG. Other provocations including inflammatory cytokines and hydrogen peroxide are just being evaluated but biomarkers have been found. This is the first global proteomic study of less abundant, LMW peptides in human placental tissue probing features of PE.

Acknowledgement: We would like to acknowledge department of chemistry & biochemistry (BYU) for funding this project.

Keywords: Lipids, Liquid Chromatography/Mass Spectroscopy, Peptides, Proteomics

Application Code: Genomics, Proteomics and Other 'Omics

Methodology Code: Liquid Chromatography/Mass Spectrometry
In children, non-invasive breath analysis is attractive, since invasive methods such as biopsies or repeated blood collection cause increased risks. Some of the 800 substances detected in human breath have already been described as potential biomarkers of physiological and pathological processes. Direct mass spectrometric techniques, such as proton-transfer-reaction time-of-flight mass spectrometry (PTR-ToF-MS) allow direct and time-resolved analysis of substances in the ppbV to pptV range.

In this study, breath gas of pediatric patients with renal diseases (n = 60), such as haemolytic uremic syndrome or kidney dysplasia, and healthy pediatric controls (n = 60) was analyzed by means of PTR-ToF-MS. Alveolar phases of exhalation were identified through breath resolved MS analysis.

More than 100 VOCs were detected in the breath of nephrological patients and controls. Data were analyzed by means of PCA and subsequent statistical testing. Isoprene concentrations were higher in nephrological patients (105 ppb vs 72 ppb) potentially reflecting hemolysis in kidney disease. Median pentanal and 2-butanone concentrations were elevated in nephrological patients (10.0 ppb vs 5.8 ppb and 0.99 vs 0.59, respectively) mirroring increased oxidative activity in renal disease. Concentrations of exogenous toluene were significantly higher in nephrological patients (1.8 ppb vs 1.1 ppb) probably indicating contact with medical equipment. In contrast, gender did not make any difference.

Breath biomarkers indicate differences between nephrologically ill and healthy children. Non-invasive breath tests may help to understand basic mechanism of the disease and in a perspective allow monitoring and tailoring therapy in pediatric patients.
Hookah smoking has been popular in the Middle East and surrounding regions for ages and its use is emerging rapidly in Western cultures making it imperative to study various aspects that may result in harm to the smoker, such as toxic trace metals, PAHs, etc. Many studies have been performed on cigarette, cigar, and pipe tobaccos while virtually no studies for these toxins have been performed on hookah tobacco. It is well documented the other tobaccos are known to contain toxic metals such as As, Cd, Cr, and Pb. However, little is known about the metal content in hookah tobacco formulations (e.g. tobacco, glycerin, honey and flavorings. Charcoal, the heat/combustion source in hookah smoking is far different than burning cigarettes and cigars. Experiments show that some charcoal brands may have as high as 10 µg/g of arsenic, making an in depth study on charcoal imperative to assess not only total metals, but metal speciation (forms), particularly for toxic metals.

Microwave assisted digestion in combination with ICP-MS was utilized to elucidate the toxic metal content in both charcoal samples and in an array of different brands/flavors of hookah tobacco smoke. Polycyclic aromatic hydrocarbons were extracted from hookah tobacco smoke using an organic solvent and analyzed by HPLC fluorescence detection. While the Arsenic totals are thought to be unacceptably high, clearly we must investigate which forms of Arsenic are present. Hence, a speciation study was undertaken to assess which inorganic or organoarsenicals are present in the neat charcoal.
Application of In Vivo and In Situ Solid Phase Microextraction to Development of Chemotherapy Regimen in In Vivo Lung Perfusion Model

Lung is often the only site of spread from malignancies such as sarcomas and colorectal carcinomas. Local high dose chemotherapy is a very attractive strategy to target therapies at the tumor site while avoiding undesirable systemic side effects of the drugs. Techniques of isolated in vivo lung perfusion (IVLP) were already shown to fulfill the expectations, however, dosing regimen and time of chemotherapy delivery should be carefully optimized to avoid local toxicity and ensure effective treatment in the same time. The main analytical challenge related to such clinical study is lack of the tool permitting non/low invasive monitoring the concentration of administered chemotherapeutics over the time of IVLP as well as distribution of the drug in the lungs with no harm to the organ. Currently available sample preparation methods for tissue analysis are mainly based on the biopsy and therefore are too invasive to be used repetitively over the period of IVLP treatment. Moreover, as the biopsy is taken from the edge of the lungs this may not reflect level of the drug inside the lobes with the course of IVLP.

In vivo solid phase microextraction (SPME) addresses the abovementioned issues. In this work we used in vivo and in situ SPME for determination of concentration of the chemotherapeutic agent in lung of living pigs and in human lungs obtained from the deceased donors, during IVLP treatment. This results showed feasibility of the method to provide fully quantitative data with good spatial and temporal resolution for the given clinical setup. The optimized method was successfully validated according to FDA guidelines as well as compared with liquid-liquid extraction results. The data showed differences in concentration profiles of DXR after administration of three different doses of the drug as well as differences in drug level in different lobes of the lungs.

Keywords: Biomedical, Clinical/Toxicology, Liquid Chromatography/Mass Spectroscopy, Sampling
Application Code: Biomedical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Assessing human exposure to chemical warfare agents remains challenging due to low exposure concentrations and complex biomedical sample matrices. We are developing sensitive methods to detect exposure to organophosphorus nerve agents by analyzing blood samples for persistent protein adduct biomarkers. Detection of nerve agent exposure is critical for forensic investigations of alleged use and confirming the identity of the agent(s) used.

Advanced sample preparation procedures and mass spectrometric measurements were used to detect in vitro exposures of human blood, plasma, and serum to the nerve agents sarin (GB), soman (GD), and VX. Butyrylcholinesterase (BuChE) is a freely circulating blood protein that is inhibited by nerve agents, making it a useful analytical biomarker for retrospective assessment of exposure. Sample preparation employed immunomagnetic separation to isolate BuChE from the plasma/serum matrix. The purified BuChE was then enzymatically digested into peptides using pepsin, which yielded nine amino acid peptides (nonapeptides) that are specific for the BuChE adduction site. Each nerve agent-nonapeptide adduct has a unique chemical structure that was separated and specifically detected using high-performance liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). Method detection limits were sensitive enough to monitor realistic exposure levels, and nerve agent-BuChE adducts were stable at 4°C for at least 26 days. Various plasma anticoagulants were also investigated to determine the effect on nerve agent exposure detection using this method. Detection of persistent protein adduct biomarkers is a promising approach for assessing nerve agent exposure in human biomedical samples.

Prepared by LLNL under Contract DE-AC52-07NA27344.
Breath VOC biomarkers (BVOCs) hold promise as noninvasive diagnostic tools of the future. Concentration of BVOCs may depend not only on biochemical or pathologic processes but also on physiological maneuvers. As breath sampling may be done in different body positions, we investigated the effect of the sampling position onto BVOC concentrations by means of real-time mass spectrometry.

An online PTR-ToF-MS was used in 15 healthy subjects for breath resolved measurements with parallel monitoring of hemodynamic parameters and pET-CO2. Paced breathing (12/min) for a total of 8 minutes in a series of different body positions (sitting, standing, lying and sitting again) was applied. Each position was held for two minutes. PTR-ToF-MS 8000 in a continuous side stream mode (sampling flow: 20ml/min, time resolution: 200ms) was used. VOCs were quantified in inspired and alveolar air by means of a custom made data processing algorithm.

Normalized mean concentrations of isoprene, furan and acetonitrile decreased by 24%, 26% and 9%, respectively, during standing and increased by 63%, 36% and 10% during lying mirroring time profiles of stroke volume and pET-CO2. In contrast, acetone, benzene (exogenous) and H2S (originating from oral bacteria) concentrations remained almost constant. During analysis in the final sitting position, all concentrations returned to initial values.

Exhaled blood borne BVOC concentrations changed due to body postures. Changes depended on cardiac stroke volume, on origin and physico-chemical properties of the substances. Patients’ positions and cardiac output have to be controlled when concentrations of breath VOCs are to be interpreted in terms of biomarkers.
The objective of this project is to develop a method for enhancing identification and quantitation of amino acids in human tears using capillary electrophoresis with LED induced fluorescence (CE-LIF). Abnormal concentration of amino acids in tears could be an indicator of eye disorders. While there are literature references of amino acids analysis via liquid chromatography coupled with mass spectrometry, CE-LIF is a much more affordable technique, and more appropriate for nanoliters tear volumes available. However, the separation of various amino acids from human tears is problematic due to complexity of tear film matrix that has specific pH, salinity, and interfering components like proteins, peptides, and lipids. A sample preparation method in our lab has been developed for sampling and derivatizing tears using solid cotton thread supports, sequential elution and CE-LIF analysis. Previous work showed most tear amino acids peaks are poorly resolved, clustered together near a dominant taurine peak and below the limit of detection of CE. In this work various sample preparation and separation procedures were studied to improve resolution and detection efficiency of the clustered amino acids. Elution of amino acids was optimized with phosphate buffer of various concentrations, pH, and elution volume portions to adjust sample salinity and recovery. To enhance CE separation, pure organic solvents and organic-aqueous binary mixtures of borate buffers with methanol, ethanol, acetone, acetonitrile, DMSO were studied. Elution recovery and subsequent signal/noise was improved using a 5 step elution procedure. Standard amino acids clustered in previous work including alanine, taurine, glycine were successfully resolved at resolutions 1.7 and with a 35-45% DMSO containing, 10-20 mM borate run buffer. Real tear samples show at least five amino acids formerly clustered near a dominant taurine peak to be resolved with a resolution 1.5 and with a signal/noise above three during a separation time of under fifteen minutes. The improved determination of tear amino acid content in tears with CE-LIF underscores the utility of the use of a thread collection method and this technique could become an affordable means for diagnosis of eye health.

Keywords: Amino Acids, Bioanalytical, Biological Samples, Capillary Electrophoresis
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Glutathione (GSH) is a major antioxidant in all animal systems. Cysteine (cys) is a precursor to GSH that plays a strong role in regulating oxidative stress in any system. Detection of these molecules usually involves a chemical reaction with free sulfhydryl functionality and is complicated by disulfide bond formation and separation of individual thiols from a chemically complex matrix. In this study, a method was developed to measure concentrations of thiols in the reduced/monomer and oxidized/disulfide forms along with glutamate, in a 20 nL hemolymph volume from a single adult [i]D. melanogaster[/i] by CE-LIF. Collection was performed using previously described methods using a capillary to collect exuded hemolymph via capillary action. Defined 10mm lengths of 50/360 I.D./O.D. fused silica capillaries were utilized to quickly and easily handle nL samples. Sample preparation involved defined capillaries to meter appropriate nL volumes of reagents including: a thiol specific labelling agent, monobromobimane (mBBr), a disulfide bond reducer, tris-2-carboxyethyl-phosphine (TCEP), and a primary amine tag, fluorescamine for glutamate content.

Capillary electrophoresis separations were performed following each reagent addition. First, derivitization with mBBr was performed to obtain free thiol concentrations. The second CE separation following the addition of TCEP generated total thiol concentrations. Lastly, derivitization of primary amines was accomplished by the addition of fluorescamine. With this assay, we were able to quantify hemolymph monomer and dimer forms of GSH and cys, plus, glutamate. [i]D. melanogaster[/i] wild-type and cystine-glutamate transporter mutant were studied with this method. Wild-type males exhibited 82.4% higher cystine levels and 59.1% higher glutamate levels when compared to mutant males. This method was also applied under oxidative stress conditions. No statistical differences for GSSG were seen for males or females under either conditions. Wild-type females under oxidative stress had the largest levels of GSH which were 34.2% higher than male controls and 69.5% higher than female mutants. This is a method that allows us to explore thiol concentrations in a biological model and can be applied to any system for oxidized and reduced forms of thiols.

Keywords: Amino Acids, Bioanalytical, Biological Samples, Capillary Electrophoresis
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Among the methods applied to peptide analyses, capillary electrophoresis (CE) provides high separation efficiency and low cost. The driving force of capillary electrophoresis is electroosmotic flow (EOF), which may be affected by many factors related to the capillary surface chemistry. As a result, very often, CE separation of peptides suffers from the adsorption of analytes on the negatively charged capillary wall, leading to poor analysis reproducibility and poor separation resolution. The goal of this research is to achieve an efficient electrophoretic separation of peptides in an aqueous solution. Our first step was to stabilize and modify EOF making it suitable for peptide separation. Three different cationic additives, including hexadecyltrimethylammonium bromide (HTAB), cetylpyridinium chloride (CPC), and triethanolamine (TRI), were dynamically coated onto the capillary wall, resulting in a reversed EOF due to the nature of cationic molecules. Electrophoretic separation was performed using a 52 cm capillary, a 20mM sodium phosphate buffer, and at 200-300 nm detection wavelength. Preliminary data show that 0.5mM HTAB, 0.5 mM CPC, and 4% (w/v) TRI enhance the separation of Caffeine and Benzoic Acid at pH 6-10. Separation was absent at pH 3 and poor at pH 4-5. The results of peptide CE separation using cationic additives will be presented, as well.

This project is supported by California State Polytechnic University, Pomona and the Goldstein Student Research Fellowship at California State Polytechnic University, Pomona.

Keywords: Capillary Electrophoresis, Electrophoresis, Environmental/Biological Samples, Peptides
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Monitoring Amino Acid Secretions from Islets of Langerhans

Gamma-aminobutyric acid (GABA) is hypothesized to play a critical role in the viability of islets of Langerhans, the endocrine portion of the pancreas. It is also thought to play a paracrine role in controlling insulin release from human islets, helping control glucose homeostasis. However, the secretion profile of GABA and other amino acids from human islets remains unclear. The objective of this project is to monitor GABA and other amino acids secreted from human islets in a time-resolved manner.

Amino acids and an internal standard, D-Norvaline, were derivatized with naphthalene-2,3-dicarboxaldehyde and separated and detected on a commercial capillary electrophoresis (CE)-laser induced fluorescence system. Derivatization conditions in the presence of the high salt buffer that islets are held in were optimized, including temperature and derivatization time. Ideal conditions were found at 25 [degree]C and 10 min. The CE separation was also optimized for resolution of the largest number of amino acids and optimal conditions were found on a 25 μm i.d., 50 cm long capillary with a separation voltage and temperature of 29 kV and 25 [degree]C, respectively. Under optimized conditions, 13 amino acids were quantifiable with limits of detections between 1-10 nM. Using the internal standard, the %RSD of the peak area ratios were between 8-12%.

Amino acids were successfully quantified from islet secretions with this method. As the glucose concentration increased from 3 to 20 mM, GABA secretion reduced from 1.9 pg islet⁻¹ min⁻¹ to 1.0 pg islet⁻¹ min⁻¹. In the future, this method will be used to produce time-resolved secretion profiles of amino acid release from human islets of Langerhans.

This work was funded in part by NIH Grant DK080714.

Keywords: Amino Acids, Capillary Electrophoresis, Optimization, Separation Sciences
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Micro free-flow electrophoresis ([micro]FFE) is a separation technique that holds promise for the simultaneous purification, concentration, and separation of proteins. In [micro]FFE, analyte streams are deflected laterally in a planar flow channel by a perpendicularly applied electrical field. Traditionally, this method has been done in an all glass device. Introduction of 3D printing will allow for simplified production and interface to mass spectrometry.

Currently there is no method for introducing samples into a mass spectrometer from a [micro]FFE device. We have investigated capturing proteins onto an intermediate membrane as they exit the separation channel of a 3D printed open-edge [micro]FFE device for MALDI-TOF analysis of the membrane. The potential benefits of this application for proteomics include reduced time and sample complexity, protein capture, concentration, and purification.

A novel 3D printed [micro]FFE device has been developed and characterized. Optimization of the device was done using fluorescently labeled dyes and laser induced fluorescence. Multiple proteins have been desorbed from PVDF membrane by MALDI-TOF after vacuum spot deposition of the proteins on said membrane. This research was supported by NSF Grant # 1152022.

**Keywords:** Electrophoresis, Lab-on-a-Chip/Microfluidics, Mass Spectrometry, Proteomics

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Western blotting is a highly valued method for protein detection and relative quantitation in complex samples. Despite the utility of the method, it is also characterized by long analysis times, manual operation, and lack of established miniaturized counterpart. We have developed a miniaturized SDS-protein size separation based separation in a microfluidic chip and direct blotting onto a moving membrane, which allows multiple separation traces to be captured on PVDF membranes in minutes comparing to hours of separation and transfer in a typical Western blotting. The system also allows multiple injects from a single sample so that multiple proteins can be probed, to improve information content from a limited sample, or obtain replicate assays for better quantification. In recent work, we have improved resolution, detection limits and throughput. Sieving media, channel dimensions, and sample injection/loading method have been optimized allowing resolution of proteins with just 2 kDa difference to be resolved. Such resolution can be obtained using pinched injection and a 4 cm long channel. Detection limit has been improved by both isotachophoresis and field amplified sample stacking. Throughput has been improved by both decreasing dimensions, allowing faster separations, and developing microfluidic manifolds that allow up to 7 parallel separation channels on one chip. Since each channel can be interfaced to 3 different samples, a total of 21 assays can be finished in 31 min.

This work was supported by NIH 1R43GM112289-01

Keywords: Capillary Electrophoresis, Immunoassay, Lab-on-a-Chip/Microfluidics, Separation Sciences
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Chromatin immunoprecipitation (ChIP) is the gold standard for probing epigenetic protein-DNA interactions that plays critical roles in cell fate and function, aging and carcinogenesis. While powerful, traditional ChIP protocols require a large cellular input (10^6-10^7 cells), which limits their utility to study biopsies, rare cells such as cancer and stem cells, and to assess tumor heterogeneity. ChIP is also laborious, time-consuming, and highly user-dependent. To address these disadvantages, we have developed an automated, droplet-based microfluidic platform that integrates cell lysis, highly controlled enzymatic chromatin processing, and magnetic immunocapture. The end result is purified nucleosomal DNA appropriate for analysis via qPCR or next generation sequencing. The reduced dimensions of the microfluidic device greatly reduce the amount of input sample required and the droplet-based platform greatly reduces material loss caused by sidewall adsorption. This enabling device will provide unprecedented opportunity to study epigenetic profiles down to the level of single cells allowing both the assessment of cell heterogeneity within complex clinical samples and the application of cost-effective epigenetic testing to very small samples in individualized medicine settings directly at the point of care.

This work is supported by Mayo-Illinois Alliance and NIH-sponsered Midwest Cancer Nanotechnology Training Center.
Plasma-Etched Cavity Carbon-Fiber Microelectrodes for Use with Fast-Scan Cyclic Voltammetry (FSCV)

Fast-scan cyclic voltammetry (FSCV), coupled with carbon-fiber microelectrodes, is a powerful tool for measuring real-time chemical dynamics in live tissue as well as at single cells. This approach offers precise temporal and spatial resolution with electrodes typically fabricated in a cylinder or disk geometry. The cylinder geometry exhibits greater sensitivity due to a larger electroactive surface area, whereas the disk geometry offers greater spatial resolution. However, several applications that require sampling from a small area would benefit from enhanced sensitivity. Herein, a novel plasma-etching procedure is described for creating carbon-fiber electrodes in a cavity geometry with precise control of cavity depth. These cavity electrodes exhibit enhanced sensitivity in brain tissue compared to disk electrodes, despite similar overall dimensions. This arises from the heightened surface roughness of the etched carbon-fiber, as studied via SEM, as well as restricted diffusion of analyte away from the electrode surface. This approach is simple and inexpensive, and a significant step forward for electrochemical experiments at preparations including, but not limited to, brain slice experiments.

Funding providing by NSF GRFP Support and Department of Chemistry, North Carolina State University

This material is based upon work supported by the National Science Foundation Graduate Research Fellowship under Grant No. DGE-1252376. Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors(s) and do not necessarily reflect the views of the National Science Foundation

Keywords: Electrochemistry, Electrodes, Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Fast-scan cyclic voltammetry (FSCV) is commonly used to perform high temporal resolution electrochemical measurements in biological systems. FSCV is superior to other chemical measurement techniques for this application because it can monitor rapidly changing concentrations of neurotransmitters in real time. Currently, carbon-fiber microelectrodes are the gold-standard probes for FSCV measurements. However, these sensors are restrained to a cylindrical or disk geometry with a diameter dictated by the manufacturer. In this work, chemical vapor deposition is used to synthesize biocompatible, thin-film polymer electrodes composed of poly (3,4-ethylenedioxythiophene) :tosylate (PEDOT:tosylate) which are capable of performing rapid electrochemical measurements. Similar films have been synthesized previously, but high surface resistivity and capacitances have limited the temporal resolution of electrochemical measurements. A low-pressure microwave-generated plasma was used to dry etch the films forming patterned electrodes with micrometer scale features of any desirable geometry. Thickness of the polymer layer and the fidelity of the etched features were characterized using optical and atomic force microscopies. The resulting PEDOT:tosylate electrodes were electrochemically characterized using steady-state cyclic voltammetry and were found to have sufficiently low resistivity and capacitance for use in FSCV. The utility of these electrodes is demonstrated by measurement of redox active neurotransmitters at scan rates 100 V/s. Further, the biocompatibility of this material is demonstrated through cell-viability assays using PC-12 cells.
Acetylsalicylic acid (ASA), known as Aspirin, is one of the oldest and most commonly used versatile and effective pharmaceuticals. After swallowing Aspirin, ASA is quickly hydrolyzed to salicylic acid (SA), one of Aspirin’s major metabolites. SA is responsible for pharmacological actions such as non-steroid anti-inflammatory, antipyretic and analgesic action in the body. The salicylates, ASA and SA, are not to just deal with many minor illnesses such as the ubiquitous inflammation symptom, fever, and pain reliever, it is has recently been used for curing and/or prevent a wide range of serious conditions such as Alzheimer’s, cardiovascular diseases and cancers including breast and colon cancers. Interestingly, a few studies indicated that high-dose aspirin may protect against catecholaminergic neuronal damage in the brain involved in diverse neurological and psychiatric disorders such as Parkinson’s disease and Alzheimer’s dementia. Dopamine and norepinephrine, the major catecholamines in the brain, have been implicated in a variety of physiological functions and behavioral responses. Despite chronic application of aspirin, an overdose can cause unwanted toxic effects including damage of mucous membrane in stomach and ulcer, its effect on catecholamine systems in the brain has not been studied yet. Here, we present effects of acute and chronic administration of the salicylates on catecholamine signaling in rat brains. The neurochemical features of catecholamines are characterized using in vivo fast-scan cyclic voltammetry with a carbon-fiber microelectrode. Our voltammetric and pharmacological evidence suggests for the first time that high-dose aspirin may alter catecholamine signaling in the brain, which is associated with some well-known side effects of aspirin.
Fast-scan cyclic voltammetry (FSCV) has found widespread use for in vivo monitoring of fluctuations in extracellular levels of catecholamines (e.g. dopamine and norepinephrine) and other electroactive neurotransmitters. Due to the high scan rates used in FSCV, accurate monitoring of the analytically relevant faradaic signal requires that the large non-faradaic charging current be stable over time such that its contribution can be removed through digital subtraction. However, gradual changes in this background current (i.e. drift) have limited FSCV measurements to short time intervals (30 - 90 sec), while microdialysis studies suggest that physiologically relevant changes in the extracellular neurotransmitter concentrations can take place over longer intervals. Previous approaches (i.e. chemometrics and analog background subtraction) have been able to mitigate the effects of drift; however, such techniques necessitate the capture of pure drift voltammograms, which poses a number of experimental difficulties in vivo, and can require specialized instrumentation.

To explore electrode conditioning as an alternative means of minimizing drift, the effects of conditioning parameters on the background drift rate and electrode characteristics were investigated both in vitro and in vivo. Using flow-cell analysis, the influence of the anodic waveform limit, cycling frequency, scan rate, and solution composition were systematically studied. Subsequently, in vivo data was collected in anesthetized animals during basal conditions and after drug challenge to evaluate the performance of an optimized conditioning protocol. Preliminary results suggest that the amount of time spent at anodic potentials where oxidative etching of the carbon fiber surface occurs primarily dictates the observed drift rate, with extended etching times resulting in small, slow-changing drift rates that allow for the possibility of longer-duration analysis.

Keywords: Electrochemistry, Microelectrode, Neurochemistry, Voltammetry

Application Code: Neurochemistry

Methodology Code: Electrochemistry
Rationally Tuning Ionic Interaction for Improving the Selectivity of Biosensors

As one kind of intermolecular interaction, ionic interaction is the most popular phenomenon in nature and plays an important role in the biosystems. The main interaction force between ions (i.e., interionic interaction) includes coulombic force, ionic-hydrogen interaction, cation-... and so on. By rationally designing the structure of ions and tuning the interactions between ions, various kinds of excellent fluorescent and electrochemical sensors have so far been developed. Our recent focus has been on the improvement of selectivity of some recognition units (e.g., enzymes and aptamers) by rationally designing and tuning the interionic interactions.

We have developed the ferricyanide-based second-generation biosensors for selective in vivo measurements of neurochemicals based on regulation of redox potential of ferricyanide mediator by carefully controlling the different adsorption ability of ferricyanide and ferrocyanide onto electrode surface.

More recently, we have demonstrated a novel dual recognition unit strategy (DRUS) to construct highly selective ATP biosensors by combing the recognition ability of aptamer towards A nucleobase and of polyimidazolium towards phosphate. Compared with the traditional strategy only with one recognition unit, the DRUS effectively improves the selectivity of ATP aptamer biosensor with a high selectivity against 100-fold higher concentrations of ADP and AMP. The constructed biosensor based on DRUS can be used for sensing the extracellular ATP concentration in the cerebral system by combining in vivo microdialysis technique and offers a promising neurotechnology to understanding brain function activity. The recognition ability of the second recognition unit (i.e., polyimidazolium) and the mechanism for high specificity are proposed and discussed.

Keywords: Bioanalytical, Biological Samples, Electrochemistry, Monitoring
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Histamine (HA) and serotonin are important neuromodulators. Histamine in the brain is involved in many brain systems such as the sleep/wake cycle, control of pituitary hormone secretion and cognitive functions. Serotonin is mostly known for regulating mood. Altered levels of 5-HT and HA in the brain are implicated in Alzheimer’s and Parkinson’s diseases and depression. Inverse modulation of 5-HT through the HA H3 receptor in the substantia nigra pars reticulate (SNr) has previously been speculated, but 5-HT and HA modulation during the treatment of brain diseases is not well understood due to the lack of ability to measure both neurotransmitters [i]in vivo[/i] in real time. In this work, we employ fast scan cyclic voltammetry (FSCV) at carbon fiber micro electrodes to study how HA and 5-HT modulate one another. We describe a novel electrochemical waveform that can simultaneously monitor the Faradaic processes of HA and 5-HT [i]in vivo[/i] in mice. We demonstrate that robust [i]in vivo[/i] detection of 5-HT and HA using FSCV can be used to effectively study and characterize the chemical profiles of the 5-HT-HA modulation in several regions in the mouse brain.
The Nano-Interface between Two Immiscible Electrolyte Solutions (ITIES) provides a unique analytical platform for the study of ionic species of biological interest such as neurotransmitters on living bio-structures. A typical nano-ITIES consists of nanometer sized pipette that can be filled with an immiscible solution for its immersion into biologically-relevant fluids. Upon electrochemical polarization, charged neurotransmitters of interest can be transferred from one phase to another, which is the basis for quantitative ionic species sensing. This is particularly useful for the detection of non-redox active neurotransmitters, especially those whose detection on carbon microelectrodes is challenging. Nano-ITIES probes thus possess significant advantages over the state of the art carbon microelectrodes, which need to be enzymatically modified, for the detection of neurotransmitters such as acetylcholine. In addition, nanopipette probes have significantly improved spatial resolution compared to microelectrode.

We studied the detection of several neurotransmitters that pose a significant analytical challenge for state-of-the-art carbon microelectrodes. These include acetylcholine, serotonin and tryptamine. Our results show that our nanopipette probes, with a typical diameter of 30 nm, can detect these neurotransmitters both qualitatively and quantitatively, with excellent signal to noise ratios and in biologically-relevant fluids. Finally, our nanosensor probes were further explored in the imaging of biological nanostructures e.g. synapses on neuronal models from Aplysia californica. Scanning Electrochemical Microscopy with nanometer spatial resolution was used for accurate positioning and imaging of neurotransmitter flux from these neurons. The strategy presented here will provide an unprecedented view on the mechanisms of neurotransmission.

Acknowledgement: This research was supported by the National Institutes of Health under Award Number R21NS085665.
Improved Understanding of Voltammetric Electrode Response Dynamics to Dopamine

Fast scan cyclic voltammetry is one of very few analytical techniques which can easily make chemical measurements with submicrometer spatial resolution and subsecond temporal resolution. As such, it has been heavily exploited, in conjunction with carbon fiber microelectrodes, to measure the spatiotemporal dynamics of redox-active catecholamine neurotransmitters in living brains. Understanding the details of these dynamics is critically important to understanding brain function on a mechanistic level. For a long time, a feature known as “hang-up” has been observed in the case of FSCV measurements of dopamine, this being a failure of the signal to return to the original pre-measurement baseline. We explain this signal feature as a consequence of a ubiquitous slow electrode response, which can be characterized by kinetic rate constants. This slow electrode response occurs in addition to the much kinetically faster and well known process of dopamine adsorption to carbon fiber microelectrodes in FSCV measurements. Our data do not support the hang-up feature as corresponding to any bulk [DA] concentration at the electrode. As such, we can improve the accuracy of our measurements of [DA] dynamic changes by treating it as a measurement artifact and removing it from our profiles of measured concentration versus time. We have developed an algorithm to do this, and set conditions which must be true for the correction process to be perfectly accurate. Violation of these conditions worsens the accuracy of correction. Accounting for the hang-up feature improves the correspondence of the FSCV signal to the [DA] concentration over time and can lead to different conclusions about the results of experiments than if the FSCV signal towards the end of the measurement were taken to closely correspond to a bulk [DA] concentration, as it appears to.

Keywords: Calibration, Electrochemistry, Neurochemistry

Application Code: Bioanalytical
Methodology Code: Electrochemistry
Liquid chromatography (LC) coupled with UV-absorption detection is a prominent analytical laboratory technique due to its broad applicability, high resolving power, and versatility. LC would be very useful for on-site environmental and clinical analysis, since measurements immediately after samples are collected would help in obtaining rapid and reliable results. Despite its popularity and wide use in the laboratory, portable LC systems are scarce. The main reasons for this are difficulties encountered in mobile phase use and disposal in the field, and re-engineering the high pressure pumping system and most commonly used detector (i.e., UV-absorption) to acceptable size, weight, robustness, and power usage.

In this work, battery-operated (24 V DC) nano-flow pumps, capable of high-pressure gradient generation, were integrated with a miniaturized LED-based detector (260 nm) for environmental pollutant analysis, and with a portable laser-based detector (405 nm) for the diagnosis of hemoglobinopathies with high sensitivity. The gradient nano-flow pumps were specifically designed for capillary column use. Low detection limits were achieved due to proper light focusing, low stray light, and very low noise. Signals down to the ppb level were easily detected with a short-term noise level on the order of $10^{-6}$ AU.
We report a battery powered ion chromatograph that weighs less than 3 kg with on-board rechargeable Li-ion batteries that provide power for 10 hours of operation. It is contained in an aluminum case measuring 30 x 25 x 16 cm. Separation relies on open tubular chromatographic columns which eliminate the need for high pressure pumps, drastically reducing weight and complexity. Eluent consumption is less than 100 µL per separation. Eluent is supplied from a pressurized vessel connected to a compressed gas cylinder, similar to ones used to inflate bicycle tires, via a voltage-controlled electronic pressure controller. Flow rates are typically < 200 nL/min which allows a single 16-20 g gas cartridge to perform hundreds of separations. The flow rate is linearly proportional to applied pressure. A release valve provides for rapid reduction of pressure. Three low power miniature solenoids, which can operate up to 800 psi, provide variable volume injection capability and further reduce weight and size. Both pressure and injection times are used to control injection volume, typically in the pL – nL range, depending on the capillary size (typically 10-30 [micro]m) in ID. Typical column lengths are 30-75 cm and 100-150,000 plates/m are generated. Separation of 5 common anions takes less than 5 min, with the separation window itself being less than 2 min. The limits of detection are in the low [micro]M range. The design also includes a novel admittance detector design with full microprocessor control including GPS location data logging and storage. Additional configuration and data readout can be performed with a computer. Field performance data for operation in the Atacama Desert in Chile will be presented.
X-ray fluorescence spectrometry (XRF) is well known as a nondestructive technique and for its rapid, simultaneous, and multi-elemental capabilities at concentrations ranging from 100% down to $\mu\text{g}/\text{g}$ (ppm). Although XRF does not have the accuracy and detection limits associated with Inductively Coupled Plasma Mass Spectrometry, its portability for positive material identification, hazardous waste screening and even for cultural heritage studies makes it uniquely attractive. Most portable XRF devices perform quantification based on fundamental parameters modeling that is specific to different modes of analysis. The primary goal of this study was to validate a “High Definition” X-Ray Fluorescence (HD-XRF) instrument developed specifically for field measurements related to assessing personal exposure to toxic metals through food, cosmetics, medicines and personal care products. Three HD-XRF units (HD-Mobile) were obtained from X-Ray Optical Systems (XOS), East Greenbush NY: one was an early prototype unit, the other two were full production models. The HD Mobile is a monochromatic micro XRF spectrometer that includes Doubly Curved Crystal optics to enhance measurement intensities. The unit is housed in a self-contained case for use in the field. Because the main use of the HDXRF is for analysis of samples with a light matrix, the plastic mode was used for quantification. Parameters such as accuracy, precision and detection limits were characterized in a laboratory setting using reference materials and standard solutions including: IAEA-413 Algae, NRC-CNRC TORT-2 Lobster Hepatopancreas, NIST SRM 1571 Orchard Leaves, IRMM BCR-627 Tuna Fish, NIST SRM-2976 Mussel Tissue, NRC DORM-2 Dogfish Muscle and IRMM ERM-CE464 Tuna Fish. Percent bias for the mean of elements such as As, Cd, Hg and Pb between days ($n = 5$) ranged from -9% to 10% for the prototype and -15% to 14% for the two production models. Five archived samples including herbal medicine products, ethnic spices and cosmetic products were analyzed with all three HDXRF units in the laboratory to compare their performance with “real” samples. Agreement between the prototype and two production models proved to be fit-for-purpose for the majority of the samples. The presence of the elements of interest as detected by HDXRF was confirmed by ICP-OES analysis of archived samples. In conclusion, these HDXRF units were shown to be suitable for the rapid screening of personal exposure to metals in the field.

Keywords: Elemental Analysis, Environmental/Biological Samples, Reference Material, X-ray Fluorescence
Application Code: Environmental
Methodology Code: X-ray Techniques
Development of a Solar-Powered, Microcontroller-Based, Remotely Deployable Potentiostat for In-the-field Electrochemical Analysis of Heavy Metals

The concept of a remote chemical analysis system implies little to no user intervention after deployment. Additionally, inexpensive microfabricated sensors combined with low power instrumentation offer unique advantages for electrochemical sensing in the field. In order to combine these advantages, and to eliminate hands-on steps such as calibration and sample pretreatment, our group is developing a coulometric analysis three electrode cell that exploits Faraday’s first law of electrolysis. In the stopped-flow, constant volume system, experimental studies using a novel coulometric stripping technique have produced linear relationships for the determination of Cu and Hg (LOD approximately 1.1 picomoles) that do not require a calibration step as a result of the known constant volume of the thin-layer cell (1.8 [micro]L).

The system instrumentation utilizes a microcontroller-driven, custom potentiostat with programmable gain control (sensitivity ranges between 0.1 nA/V and 100 nA/V) and automated sample loading using DC powered pumps/valves connected in-line with the sample and buffer flow paths (programmable flow rates of 1-1000 [micro]L/min). The microcontroller software under development allows for on-demand or periodic experiments (10 – 20 times per day, for example), local storage of experimental data (i.e. to microSD memory card) for later retrieval, and switching into low-power sleep mode between measurements. The software also periodically interrogates the potentiostat using a built-in dummy cell as a self-check. Additional features include a 12V (15 W) solar panel to charge a 2200 mAh lithium-ion battery that powers the system (approximately 35 mA current draw during a 60 sec experiment). A low-noise virtual ground technique is used to establish a split Vdd range of ±5V that is used to power the instrumentation amplifiers included in the potentiostat circuit. Ongoing development includes lowering the noise floor for higher sensitivity, reduction of sleep-mode power consumption, and incorporation of a GSM modem for periodic transfer of data over existing cellular infrastructure.

Keywords: Electrochemistry, Environmental/Water, Portable Instruments, Stripping Analysis
Application Code: Environmental
Methodology Code: Electrochemistry
A New Method for the Analysis of ppb Levels of Mercury in Air and Water

Nonspecific methods such as UV absorbance or fluorescence have been successfully used for the analysis of ppb concentrations of mercury in air and water. The unique amalgamation of mercury with gold and silver makes this possible. Air samples are collected and only the mercury is reacted with the gold surface. Any impurities are purged through, then the amalgam is heated to desorb the mercury which is measured by the detector.

We have used a very sensitive but nonspecific photoionization detector (PID) with a 10.6 eV lamp to measure mercury that has an ionization potential of 10.43 eV. The chemistry of the gold/mercury amalgam makes this method specific for mercury. The Permissible Exposure Limit (PEL) for Hg is 0.05 mg/m³ (0.408 ppm). Mercury permeation tubes were used to generate levels from 0.02 to 1 ppm. Preliminary data indicates a detection limit of > 10 ppb of Hg for the gold film photoionization detector (GFPID) analysis.

Water samples are analyzed using hydride generation with sodium borahydride to reduce the inorganic salts to elemental mercury that is efficiently detected by the PID. The EPA MCL for mercury in drinking water is 2 ppb. The detection limit for Hg in water by hydride generation GFPID was 0.2 ppb.

Keywords: Air, Elemental Analysis, Environmental Analysis, Water
Application Code: Environmental
Methodology Code: Other
For the last several years, we have been developing and characterizing 2d microplasmas on planar chips. More recently and to reduce fabrication costs, we used 3d-printing technology to make sugar-cube size, battery-operated microplasmas on 3d-chips.

Are these microplasmas any good for any analytical applications? To address this question and to demonstrate analytical capability and utility, we will discuss two applications. One involves the determination of the concentration of Zn in mm-long single stands of hair and the other of Pd in paint. Both of these were analysed as solids (i.e., without an acid digestion step), thus expediting analytical determinations. It is worth noting the if an ICP (Inductively Coupled Plasma) was used with a pneumatic nebulizer for sample introduction, acid digestion would be essential for both of these sample types.
Accurate and reliable detection of different gases is required in many application areas, for example in industry, indoor and outdoor air quality, health and well-being, and defense and security. Also, high sensitivity and selectivity, rapid detection, and small instrument size are common criteria, especially when monitoring compounds that are harmful to humans.

Photoacoustic detection does not require long absorption paths and therefore it is possible to combine the high sensitivity with a small-sized detector. The use of optical cantilever microphone further enhances the sensitivity of the photoacoustic detection. When compared to more common capacitive microphones, optical cantilever microphone can achieve orders of magnitude higher sensitivity [1], which opens possibilities beyond the reach of the conventional infrared techniques.

In this study, different applications of cantilever-enhanced photoacoustic spectroscopy are investigated. The applications include, for example, indoor air quality measurement of different volatile organic compounds (VOCs), unknown gas identification, food process control, and forensic use and border control for drugs and explosives. In all of these applications, multi-gas detection with high selectivity and sensitivity is required.

Cantilever-enhanced photoacoustic cell was combined with a tunable infrared source, either an optical parametric oscillator (OPO) or a quantum cascade laser (QCL), to be able to scan the photoacoustic infrared spectrum and perform multi-component analysis. As QCLs and OPOs are becoming more common, they are available in greater number of wavenumber ranges, higher optical output powers, and are more and more affordable. Ppb and sub-ppb level detection limits were achieved with the combination of cantilever-enhanced photoacoustic cell and tunable infrared sources.


Keywords: Air, Photoacoustic, Spectroscopy, Trace Analysis
Application Code: Safety
Methodology Code: Vibrational Spectroscopy
The determination of tertiary alkyl amines using modern chromatography instruments has proven to be highly challenging due to the sticky nature of these compounds which causes significant issues with carry-over. Structural properties of the tributylamine (TBA) molecule were correlated to its problematic interactions within the flow path of the Agilent 7890A Gas Chromatograph and 5975C Mass Spectrometry Detector. TBA has a pKa of 10.9 and a high boiling point of ~214 °C. Though it is technically not a cation, when the nitrogen atom is protonated, it holds a strong positive dipole which ensures that it will occupy any active sites found in the instrument. Direct injections were made into a split liner with deactivated wool. An Agilent DB-5MS UI column was used for the separation due to its maximum operating temperature of 325 °C. TBA is a hydrophobic compound and the presence of having only one constricted site, at which hydrogen bonding can occur, limits the possibilities of using certain solvents for sample preparation and Solid Phase Extraction (SPE) clean-up. Samples were diluted in a pH 13 solution of ammonium hydroxide in acetonitrile. A Bond Elut CBA cartridge was used to extract TBA. To remedy the problem of carry-over, the MSD was operated in Selected Ion Monitoring (SIM) mode. A quantitative method for TBA, developed using GC-MS, is presented here. The LOQ was found to be at 0.25 ppm, and matrix spike recoveries were reproducible and between 96-115%. The average response factor RSD was reproducible, in the range of 4-8%.

Keywords: Electrochemistry, Fuels\Energy\Petrochemical, Gas Chromatography/Mass Spectrometry, Ion Exchange
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography/Mass Spectrometry
In order to more accurately model performance and critical properties of liquid fuels based on composition, detailed qualitative and quantitative information of the sample must be obtained. Currently one of the most comprehensive methods for fuel analysis is GCxGC-MS. Comprehensive GCxGC-MS couples two chromatographic columns with orthogonal selectivities to a fast Time-Of-Flight (TOF) mass analyzer. This approach enables direct analysis of complex organic mixtures providing detailed compositional information due to the inherent separation efficiency of a cryogenically modulated GCxGC. Since mass spectrometric detection is not quantitatively linear with respect to different compound classes due to differences in molecular ionization efficiencies, to obtain a more quantitative measurement, it will be necessary to establish compound class response factors. This was accomplished by configuring a secondary channel on the GCxGC-MS, such that the second column effluent is routed to a flame ionization detector (FID), which responds linearly to concentration. A three-way capillary flow splitter was installed into a GC with some modifications to the secondary column oven. This enabled the simultaneous collection of both FID and MS data channels, as shown in figure 1. This modification allows the collection of MS data that contains qualitative information about the sample compounds (compound identification) and the universal signal response of the FID will allow for analyte quantification. Quantitative MS measurements were thus obtained from a series of surrogate fuel blends with known compound abundances, and directly correlated with their FID responses. This provided the information necessary to establish response factors for each compound class. A secondary correction was also developed to account for changes in detector response with carbon number to produce class specific libraries of carbon number factors that will be used to improve fuel property modeling.

**Keywords:** Capillary GC, Fuels\Energy\Petrochemical, Gas Chromatography/Mass Spectrometry, Petroleum

**Application Code:** Fuels, Energy and Petrochemical

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Electron Ionization Gas Chromatography / Mass Spectrometry (EI GC/MS) is a powerful and information-rich technique for qualitative characterization and quantitative analysis of the compounds in a mixture. One of its most valuable functions is to provide the molecular weight of a compound. However, many compounds do not have a stable molecular ion under EI conditions and it is small or absent in the spectrum.

Cold Electron Ionization GC/MS (Cold EI GC/MS) can enhance the molecular ion abundance of most compounds while retaining the EI fragmentation pattern for spectral library searching.

The enhanced molecular ion intensity is achieved by supersonic expansion of the GC effluent into a molecular beam, where the analyte molecules are vibrationally and rotationally cooled adiabatically to ~10 - 30 K. When this vibrationally cold molecule is ionized by a 70 eV electron the final thermal energy is much lower than in EI, which ionizes a molecule at GC transferline temperature of several hundred °C. The reduced Cold EI internal energy produces much less fragmentation of the molecular ion. The ion source used in this work is of axial “fly-through” design, eliminating molecule and ion collisional scattering with ion source walls which could lead to additional fragmentation. This is demonstrated for compounds such as squalane, which shows a large molecular ion with Cold EI, but <1% by conventional EI.

When used in a novel q-TOF configuration the enhanced molecular ion increases sensitivity, selectivity, and information content.
The goal of prep chromatography is to isolate specific compounds. Each project is a balancing act for the triangle of purity, yield and throughput. When picking the stationary phase for a prep chromatography project, the first thought has to be selectivity. If the stationary phase doesn’t separate the desired material from the undesired material there is no hope for the separation. Even if there is some resolution, if the peak shape is very poor, it might be impossible to achieve the required project goals. Often pH is an effective tool for chromatography and can be used to adjust selectivity and peak shapes. Another powerful tool is using a core-shell media. Core-shell provides higher efficiency, improved sensitivity, and increased resolution when compared to what could be achieved with the same stationary phase on the same size fully porous media. The properties achieved by core-shell material will help significantly with the purity and yield aspect of a prep chromatographic method. The work presented here will demonstrate through several examples how core-shell media that can be used at high pH can be a powerful tool for the purification of pharmaceutical compounds.

Keywords: Biopharmaceutical, HPLC, Liquid Chromatography, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Liquid chromatographic methods, such as reversed-phase liquid chromatography and electrochromatography, are powerful and popular tools in the pharmaceutical industry. The past few years have seen more and more large molecules drugs, especially therapeutic monoclonal antibodies entering the market that was previously dominated by small molecule drugs.

Many important questions have to be re-considered as the analyte size changes from less than 1000 Da to 150,000 Da or more. In this presentation, an important question is addressed, which is, how accessible are the surfaces of common column materials to large analyte such as monoclonal antibodies.

We explored the answer experimentally by stacking monoclonal antibodies on the surface of three common liquid chromatography column materials, fully porous particles, core-shell particles and non-porous particles. The column materials are packed into UV-transparent capillaries under sonication to form homogenous packing. Fluorescently labeled monoclonal antibodies were injected into the packed capillaries with 5% acetonitrile in water with 0.1% Trifluoroacetic acid. Under such strong retaining condition, the IgG2s were pushed onto the surface of the packing material, forming a stacked plug. By comparing the length of the stacked plugs on different columns, we discovered that the surfaces of the core-shell particles and totally porous particles were largely inaccessible. Among the three, sub-micron non-porous particles provide the largest accessible surface area. It’s therefor suggested that larger pores (than 300 Å) are needed for the characterization of monoclonal antibodies.

We also investigated sub-micron silica colloidal crystals for the analysis of monoclonal antibodies from unwanted contaminants by electrochromatography. Beside the larger accessible surface area, the narrower size distribution and faster mass transport of silica colloidal crystals lead to lower plate heights and increased resolution. Longitudinal diffusion is reduced with the plug-like flow profile in electrochromatography.

This work is supported by NIH under Grant R01-GM101464.

Keywords: Bioanalytical, Liquid Chromatography, Pharmaceutical, Quality Control
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Method development involves investigating a range of parameters to generate conditions for optimum separation. While there are many approaches to method development such as one-factor-at-a-time (OFAT), systematical protocols, and quality-by-design (QbD), the final goal is to develop a robust method that separates all components and generates an accurate result, time after time.

The arbitrary change of one factor at a time for method development (OFAT) is easy to perform, but typically does not result in a robust method. With a systematic approach, a predefined protocol is used, which typically incorporates scouting, screening, and optimization steps. Although this approach may result in development of a reproducible method, the most reliable separation conditions may not actually be identified. Analytical QbD approaches use statistical software to generate a design of experiments (DoE) to comprehensively study interactions of different factors to define a region of operating space with high method robustness.

In this work, we compare the development of a UPLC method for metoclopramide HCl and related substances using systematic protocol and analytical QbD. Both strategies also employed an integrated UV and orthogonal mass detection workflow to help automate and accelerate the peak identification and tracking process. We will compare results from both method development approaches and demonstrate that analytical QbD offers a thorough examination of variables and provides a measure of robustness which is integral to the final method.

Keywords: HPLC, Method Development, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Tea, a popular drink with different varieties and flavors, is often considered as a medicinal alternative to fight against diseases. Empirical findings prove that tea drinks are able to provide many health benefits including antioxidation, anti-inflammatory, and neuroprotective functions. In recent years, scientists found that the key ingredients contributing to tea’s antioxidation benefits were catechins, a type of polyphenols that can eliminate free radicals inside organisms. To date, analytical methods employed high performance liquid chromatography (HPLC) have been applied to investigate copper induced oxidative stress in tea leaves and to investigate effects of water solution on extracting green tea leaves. However, many extraction (tea brewing) factors including temperature, solvent, and flavor supplement have not been thoroughly examined. Therefore, our project is to focus on determining the optimal extracting conditions for catechins from dry tea leaves and investigating how brewing parameter affect antioxidation functionality of catechins in tea drinks. Green tea extracts under different brewing conditions were analyzed by a reversed-phase HPLC method using a C18 column, and a mixture of methonal/water (20:80) as the mobile phase with a detection wavelength of 280 nm. Four major catechins in tea drinks were analyzed in 15 min. Preliminary data show that an introduction of ~0.5% NaCl to green tea drinks increases the extraction of catechin from green tea leaves. The results from the Fenton reaction assay will be presented, as well.

This project is supported by California State Polytechnic University Pomona and the Goldstein Student Research Fellowship at California State Polytechnic University Pomona.

**Keywords:** Beverage, Extraction, HPLC, Natural Products

**Application Code:** Food Science

**Methodology Code:** Liquid Chromatography
Ion exclusion chromatography (IELC) has been used to effectively separate short chain aliphatic acids, primarily carboxylic acids often found in various drinks. Separations by IELC are commonly performed on strong cation exchange columns, in which the negatively charged sulfonate groups, making up the Donnan membrane on the stationary phase, will repel the negatively charged analyte ions, but allow the neutral species to penetrate the membrane and enter the pores with the occluded mobile phase. In addition to ion exclusion and steric effects, hydrophobic adsorption on the resin must also be considered as a potential aspect of the retention mechanism. In general, carboxylic acids with higher pKa values and more hydrophobic sites show longer retention. We have characterized for IELC a reversed phase column previously coated with a sulfonated surfactant. This surfactant modified ultra high-performance liquid chromatography (UHPLC) reversed phase column allowed for flexibility and customization of the column to the needs of the separation. A Kinetex XB-C18 reversed phase HPLC column (150mm x 4.6mm id, 2.6µm) was dynamically modified with sodium dodecylsulfate (SDS) for the separation of aliphatic and aromatic acids. The mobile phase was optimized to 1.84mM H2SO4 (pH 2.60) at 0.60 mL•min⁻¹, with a 20µL injection volume with UV detection at 200nm. Under these conditions, short chain aliphatic carboxylic acid mixtures, such as geometric isomers, fumarate and maleate, were efficiently separated with baseline resolution.

Separation of aromatic carboxylic acids, such as o- and m-hydroxybenzoic acids, proved to be more challenging due to the complexity of the separation mechanism to include a strong hydrophobic effect. To improve separation efficiency of aliphatic and aromatic acid mixtures, we propose using a mobile phase modifier, such as methanesulfonic acid, or a shorter chain stationary phase modifying surfactant, such as C6 or C3.

Keywords: HPLC Columns, Liquid Chromatography
Application Code: Food Science
Methodology Code: Liquid Chromatography
The incorporation of halogen atoms into the structure of active pharmaceutical ingredients (APIs) has become very important, as it is assessed that 20 percent of all pharmaceutical drugs are halogenated. One of the most useful ways to increase the bioactivity of an API is to introduce one or more fluorine atoms in the structure. Frequently dehalogenated impurities/by-product exist as side reactions during the production of the API may result in hydrohalogenation. These dehalogenated impurities often coelute with the major product of interest and also can persist through further synthesis steps. Further, the dehalogenated impurities are difficult to separate from the API. We have investigated a number of chromatographic methods to identify optimum columns and conditions for separating dehalogenated impurities. The drugs evaluated in this study were aprepitant, atorvastatin, voriconazole, ezetimibe, paroxetine, risperidone, ciprofloxacin and ofloxacin. Cyclofructan, cyclodextrin, macrocyclic glycopeptide and zwitterionic based stationary phases were screened in the reversed phase, polar organic mode and HILIC to separate APIs from their des-fluoro analogs. Method optimization was performed for each drug.

Keywords: HPLC, HPLC Columns, Liquid Chromatography, Process Analytical Chemistry
Application Code: Process Analytical Chemistry
Methodology Code: Liquid Chromatography
Encapsulated bonding technology (EBT) is a new approach to stationary phase end capping that provides exceptional inertness and excellent phase stability across a broad pH range from 1.5 to 11. This technology has previously been successfully applied to 2, 3 and 5 micron totally porous (non-core) packing materials, but has more recently been implemented for solid-core packings. The ability to use stationary phase chemistry (C18, phenyl-hexyl), organic modifier choice, and pH as variables in a systematic approach is a significant advantage for UHPLC and HPLC method development. The usefulness of such a method development strategy will be described and demonstrated with an appropriate example.
In capillary chromatography, on-column porous frits are used to retain packed bed materials and allow for more reproducible and efficient separations. Shortcomings of current methods include bubble formation during particle sintering and irreproducible frit synthesis. Polymer frit synthesis can be initiated using UV radiation, but necessitates introduction of a fragile window within the capillary, whereas thermal radiation can be performed in the presence of the protective polyimide coating. Here, we report a new approach for frit fabrication employing thermal polymerization to form polymer frits within a fused silica capillary. Frits were synthesized in 3-(trimethoxysilyl)propyl methacrylate modified capillaries via free radical thermal polymerization using a sol-gel solution containing 2,2-azobisisobutyronitrile in the presence of glycidyl methacrylate, ethylene glycol dimethacrylate and porogenic solvent decanol. Thermal polymerization was initiated using a custom-built temperature controlled heating apparatus. Frit length was studied as a function of polymerization time and temperature. Frits synthesized at a lower temperature and longer time resulted in more stable frits compared to higher temperatures and shorter polymerization times. The breakdown pressure of frits was determined by identifying a deviation from linearity in the plot of backpressure versus volumetric flow rate. Thermal frits demonstrated similar stability compared to UV polymerized frits. Using zonal chromatography, the performance of C\textsubscript{18}-packed capillary columns was compared for thermal versus UV-polymerized frits. This method offers short polymerization times, control of frit length, control of frit placement and improved capillary stability via retention of the polyimide capillary coating.

Keywords: Bioanalytical, Capillary LC, Electrophoresis, Polymers & Plastics
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
We developed a software package entitled MetSign2 for analysis of stable isotope assisted metabolomics (SIAM) acquired on high resolution mass spectrometry. A significant feature of SIAM data is the overlap of isotopic peaks generated by isotopologues as well as co-eluting metabolites. MetSign2 first deconvolutes the mass spectra into isotopic peaks. Metabolites were then tentatively assigned based on m/z value and the isotopic peak profile. MetSign2 further deconvolutes the overlapped isotopic peaks using a linear regression model, assuming the intensity of isotopic peak is additive and can be represented by a linear combination of overlapped isotopic peaks. By solving the linear regression model, the weights (i.e. the contribution from each isotopologue) are obtained. To prevent negative values of weight, an iteratively linear regression process is employed, in which the isotopologues with negative weights are considered as noise and removed from the list of candidate isotopologues in an iteration. However, some of the deconvoluted isotopologues may be noise due to the match of their m/z values. To determine the threshold of weights obtained from the linear regression model, metabolites recorded in KEGG, LIPID MAPS and HMDB were used to generate overlapped isotopic peaks, with 2%, 5%, 7% and 10% of Gaussian white noise added to the abundance of each isotopic peak, respectively. The linear regression model was trained using these simulated data and the optimal threshold of the weight value was determined as 7.5x10-7.

The algorithm was implemented in Matlab 2010b and tested with mixtures of compound standards and biological samples. Our initial tests demonstrate that MetSign2 is able to remove [sup]13[/sup]C natural abundance contribution and uncover the true [sup]13[/sup]C enrichment in detected metabolites. Figure 1 shows the comparison result of [sup]13[/sup]C isotopologues of compound ATP between before deconvolution and after deconvolution.

**Keywords:** Bioinformatics, Data Analysis, Mass Spectrometry, Metabolomics

**Application Code:** Genomics, Proteomics and Other Omics

**Methodology Code:** Data Analysis and Manipulation
Type 1 diabetes is caused by autoimmune destruction of insulin-secreting beta-cells found in the islets of Langerhans of the pancreas. Severe cases can be treated by islet transplantation. However, islets used for transplantation can undergo a great amount of oxidative stress during the isolation process, which could have profound effects on islet viability and potency. Metabolomics provides a powerful tool for investigating stress-induced alterations in cellular pathways by enabling the quantification of a wide range and amount of metabolites. Liquid chromatography-time-of-flight mass spectrometry is a useful metabolomics technique because of its high sensitivity and selectivity.

Previous metabolomics studies have typically been conducted with clonal beta-cells due to their ease of access and specificity for beta-cell metabolism. However, because clonal cells are derived from a cancer line and as such may favor pathways involved in cell growth, corroboratory data from native islets is necessary. One challenge is that islets vary widely in size, so beta-cells within an islet may be exposed to varying concentrations of stimulants, leading to a more variable metabolic profile and potentially poor reproducibility of results.

We have developed a sample preparation method for the reproducible measurement of ~70 metabolites within whole islets through optimization of islet number, extraction method, and extraction solvent. Analyses were performed using a hydrophilic interaction liquid chromatography/anion exchange (HILIC/AEX) column or a reverse phase column coupled to a TOF-MS to measure polar metabolites and lipids, respectively. We then applied this method to study the effects of hydrogen peroxide treatment on islets as a model of oxidative stress. Several key metabolites were found to be significantly altered by this treatment. Future work involves investigating whether these metabolites can be used as markers of islet viability/potency for transplantation purposes.
Matrix-Assisted Laser Desorption/Ionization Mass Spectrometric Imaging (MALDI MSI) of Glycolytic and Oxidative Skeletal Muscles

A MALDI MSI methodology was developed to analyze and visualize the distribution of glycolytic and oxidative skeletal muscles fibers. Soleus (SOL), extensor digitorum longus (EDL), and gastrocnemius (GAS) muscles from 6-month old rats were used for this study. SOL is mainly composed of oxidative type I muscle fibers, EDL is mainly composed of glycolytic type II fibers, and GAS is composed with a mixture of both type with regionalization of oxidative fibers.

Tissue sections were obtained at 10 µm thickness at -25 °C, and thaw mounted atop glass slides. Serial sections were used for immunofluorescence staining and positive and negative ion mode MSI analyses. For positive ion mode, 0.5 M 2, 5-dyhydroxybenzoic acid (DHB) in methanol was used as the matrix, whereas 10 mg/mL 9-aminoacridine (9AA) in 70:30 (v:v) ethanol:water was used for negative ion mode. The matrix coated tissue sections were analyzed by a Thermo LTQ XL with a ~100 µm laser spot size.

Differences were observed from the comparison between the averaged mass spectra recorded from each muscle. Principal component analysis (PCA) was also performed. Separation between muscle group sample points was observed from the 2-D scores plot. The loadings plots were analyzed for significant m/z values of each sample group; m/z values with different localization were observed in MS images. In negative ion mode, fructose-bisphosphate (m/z 339) showed higher relative intensity in SOL and oxidative GAS, whereas inosine-monophosphate (m/z 347) showed higher relative intensity in EDL and glycolytic GAS. In positive ion mode, phosphatidylcholine (PC) (18:1/18:1) showed higher relative intensity in SOL and oxidative GAS, whereas anserine (m/z 241) showed higher relative intensity in EDL and glycolytic GAS. Overall, this study showed the different metabolic profiles in oxidative and glycolytic muscles, and demonstrated the ability to visualize the tissue structural information with minimal loss of chemical information.

Keywords: Imaging, Lipids, Mass Spectrometry, Metabolomics
Application Code: Genomics, Proteomics and Other ’Omics
Methodology Code: Mass Spectrometry
A highly sensitive platform was developed to determine anionic polar metabolites by coupling capillary ion chromatography with high resolution accurate mass spectrometry. This method provided separations of isobaric sugar monophosphates, sugar diphosphates, and organic acids using a capillary format anion-exchange column optimized for high resolution and efficient separations of organic acids using supermacroporous, 4-micro]m particle resin particles. The analytes were detected by an orbitrap mass spectrometer resulting in mass accuracy <1 ppm and fmole on column sensitivity.

This method was applied to metabolomic profiling of metabolic biomarkers for oral squamous cell carcinoma (OSCC) metastasis, 3-paired OSCC cell lines (UM1, UM5, CSC) with wild-type controls. Cap IC demonstrated outstanding separation and peak shape for anionic polar metabolites with increased sensitivities [greater than]100-fold as compared to RPLC and HILIC methods. Differential analysis revealed significant changes in energy metabolism pathways.
The studies reported herein describe the performance of 1-aminopyrene (AP) and AP-derived group of uniform materials based on organic salts (GUMBOS) as novel matrices for matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) analyses of peptides. A number of solid phase salts of AP, i.e. GUMBOS, are synthesized of variable hydrophobicity simply by changing the counterions. The primary aim of this study is to investigate the performance of these GUMBOS as well as AP as new MALDI matrices on the basis of their hydrophobicity for the analysis of hydrophobic as well as hydrophilic peptides. A clear trend was observed between the signal intensity of hydrophobic peptide and hydrophobicity of matrices. The hydrophobic matrix materials are more effective for hydrophobic peptides and, similarly the most hydrophilic peptide displayed great intensity in the most hydrophilic matrix. A mixture of hydrophobic and hydrophilic peptides demonstrated the variation in signal intensities in different AP and AP based GUMBOS, and these signal intensities directly depend upon the hydrophobicity of the matrix. The performance of these materials in terms of reproducibility is also compared with that of $\text{CN}$-4-hydroxyl benzoic acid (CHCA), which is a widely used matrix for peptide-mass fingerprinting in proteomics. These materials showed good signal intensity for hydrophobic peptides with good reproducibility.
Physalis angulata is a weed used medicinally for several diseases including infertility in both males and females, prevention of miscarriage and management of asthma, throat swelling, muscular stiffness and pain, rheumatism, gonorrhea, diarrhea, malaria and skin disorders. In order to correlate the constituents of the whole plant with these medicinal uses, the essential oil was extracted and analyzed by gc-ms. The whole plant was air-dried and the essential oil extracted into hexane by hydrodistillation using a Clevenger apparatus. Collection was in two modes; single collection over four hours and hourly collection over three hours. Analysis was on Agilent gc-ms (7890A/MSD 5975C) fitted with HP 5MS column using a temperature program of 80 deg.C (2min) increased at 5 deg./min to 120 deg.(2min) increased at 2.5 deg./min to 240 deg.(6min). The major constituents were tetradecanal /E-2 tetradecene 1-ol (15-16%), 2 –pentadecanone, 6, 10, 14 trimethyl (23-30%), cis-vaccenic acid (11.8-20.6%) and 9,12-octadecadienoic acid methyl ester (4.2-6.0%). Other constituents include caryophyllene oxide (2. 6%) in the first hour fraction, 9, 12, 15 octadecatrienoic acid (Z, Z, Z)- (0. 9-1. 2%) and cis/trans – 13-octadecenoic acid methyl ester (1.3-2.0%). Small quantities of phytol, hexadecanoic acid and the methyl ester were also present. Caryophyllene oxide is anti-inflammatory, antibacterial and anti-fungal, is an analgesic, exhibits repellency against malaria vector and has been used for the prevention and treatment of cancers. Phytol is anti-inflammatory and is useful in the management of arthritis and tumors and has been proposed as an active ingredient in asthma plants. Long chain unsaturated fatty acids such as cis-vaccenic acid exhibit anti-inflammatory, antibacterial and anti-fungal activity. Thus the constituents of the essential oil lend support to some of the uses of the plant in Alternative medicine.

Keywords: Analysis, Extraction, Flavor/Essential Oil, GC-MS
Application Code: Bioanalytical
Methodology Code: Gas Chromatography/Mass Spectrometry
Dogs can reliably distinguish by sniffing between healthy and ill people suffering from cancer. These obviously existing volatile cancer markers have long been searched for, but have not yet been detected.

In cancer tissue, high levels of nitric oxide (NO), nonvolatile thiols and nonvolatile nitrosothiols (SNOs, being a synthesis product of both) have been described as typical. Taking this into consideration, volatile nitrosothiols (vSNOs) generated from volatile thiols that are omnipresent in malign tissue and NO are proposed to be the source of the characteristic smell of cancer.

Derived from the structures of volatile sulfur compounds repeatedly found in the breath emission of humans (e.g. thiols, sulfides and disulfides), with typical carbon structures based on methyl-, ethyl-, 2-propyl- and propenyl- (allyl) groups, the corresponding vSNOs were synthesized and stabilized for weeks as n-decane solutions (see poster for syntheses and analyses of vSNOs).

To prove that the smell of cancer consists of vSNOs, a dog was trained with an artificial odor mixture composed of the four vSNOs structured as mentioned above. In succeeding sniffing tests, this dog was reliably able to find cancer tissue amongst non-cancer tissues without ever before having sniffed cancer. This is the first time that a dog training procedure using artificial fragrances has enabled a definite and secure detection of cancer. These results are strong hints for vSNOs as cancer markers. But their detection remains difficult due to their fast decomposition rate (e.g. during enrichment) and until now has been unsuccessful in vivo even when handled with consideration by cryo-sampling and low temperature GC.

Keywords: Gas Chromatography/Mass Spectrometry, Headspace, Organic Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Gas Chromatography/Mass Spectrometry
Detection of VOC-Patterns Emitted From Mycobacterial Cultures by Micro-Extraction-Techniques and GC-MS

Mycobacterium avium spp. paratuberculosis (MAP) is the causative agent for Johne’s disease, an intestinal infection in ruminants, which results in economic losses of at least 200 million US-dollars per year. Fast diagnostic of MAP infection is difficult because of slow growth of the pathogen. This study was intended to detect MAP specific patterns of volatile organic compounds (VOCs) in headspaces over in vitro cultures by means of two different micro-extraction techniques.

239 cultures from three different MAP-strains, grown on five different media, were examined. VOCs were extracted from headspace (20mL) of in vitro cultures by means of bidirectional needle-trap-micro-extraction (NTME, 160 samples), or solid-phase-micro-extraction (SPME, 79 samples), respectively. GC-MS was used to identify and quantify extracted VOCs. 113 VOCs, alcohols, alkanes, alkenes, aldehydes, ketones, sulfides, esters, furans and aromatic compounds were detected in ppbV-/high-pptV-levels. 51 of 96 substances detected through NTME and 18 of 82 substances detected through SPME had significantly different concentrations in cultures compared to blank media. Specific VOC patterns could be attributed to different MAP strains and to density of bacterial growth. 17 VOCs were only detected by means of SPME and 31 VOCs only by NTME. 13 of the differentiating VOCs were found by means of NTME and SPME.

VOCs emitted from MAP cultures revealed characteristic patterns. As sensitivity can be improved by increased sampling volume, NTME is better suited for comprehensive VOC-profiling. For some substances, SPME provided additional information. Smart combinations of micro-extraction and GC-MS can be used to detect and characterize mycobacterial growth in cultures.

Keywords: Bioanalytical, Biological Samples, SPME
Application Code: Bioanalytical
Methodology Code: Gas Chromatography/Mass Spectrometry
Macroautophagy is the process by which cellular debris and excess protein is degraded in living cells, maintaining homoeostasis. Dysregulated macroautophagy results in the accumulation of unnecessary protein and cellular debris, and has been linked to pathologies including myopathy and muscle wasting. Proteomics is essential to investigate the pathways behind the dysregulation of macroautophagy. Unfortunately, conventional proteomic strategies cannot distinguish between the proteomes of multiple cell types co-existing in a tissue. Mass cytometry is an emerging technology that measures cellular markers on the surface or within single cells that allows for detection and quantification of upwards of 30 separate cellular targets labeled with isotopically pure heavy metals. The instrumentation combines the high mass accuracy and low limits of detection that are characteristic of ICP-MS, with the single cell analysis capabilities of flow cytometry. Because of the shape and size of muscle fibers, this technique has not been used for the analysis of muscle fibers. In this presentation it will be shown that both intra and extracellular markers of macroautophagy, previously identified by proteomics, can be detected and quantitated in individual mouse muscle fibers, using mass cytometry. Immunofluorescence microscopy has been used to identify antibodies appropriate for use in mass cytometry. Mass cytometry data will be evaluated using SPADE analysis, and fibers with similarly expressed markers will be grouped. This analysis will allow for the discovery of relationships between cellular markers of macroautophagy. Ongoing work on this project includes labeling appropriate antibodies, and titration mass cytometry experiments.
Imaging mass spectrometry, a powerful label-free imaging technique with high sensitivity and selectivity, has been increasingly applied in biological and medical research. Drosophila melanogaster and C. elegans have relatively simple nervous systems but possess high order brain functions, many that are similar to humans. Therefore, these have been used as common model systems in biological studies. In our work, multimodal imaging techniques including ToF-SIMS and MALDI have been utilized to image the fly brain and the worm in order to investigate the effects of stimulant drugs on the brain’s chemical structure and the basic chemical anatomy of the extremely small worm.

From ToF-SIMS analyses, different distributions of various molecules, particularly eye pigments, fatty acids, diacylglycerides, and phospholipids have been found spatially across the fly brain. In C. elegans, interesting pattern of lipid heterogeneity has been uncovered. Statistical analysis of the fly data with PCA shows that the amounts of several lipids, fatty acids and diacylglycerides in the central brain are altered by drug administration. Tandem MS has been performed to elucidate the structures of molecules affected by the drug. In MALDI analyses, we have explored different sample preparation methods including the use of Au and Pt nanoparticles (NPs), and sublimation to gain improved spatial resolution and sensitivity. Low mass molecules, particularly eye pigments, diacylglycerides, and intact lipids, were detectable using Au NPs. In contrast, Pt NPs and sublimation with DHB offered the possibility to detect high mass molecules including intact lipids and peptides with m/z up to 1800 Da. Both types of NPs provided better spatial resolution (20 µm) compared to sublimation. The orthogonal SIMS and MALDI methods provide complementary chemical structural information making them very useful for biological studies on invertebrate models.
Inflammatory bowel disease (IBD) is a widespread GI tract disease. It affects 1.4 million people in the US and 2.2 million people in Europe. IBD can be triggered by an abnormal immune system response. The exogenous antigens activate T-cells, releasing cytokines from the intestinal epithelium. These cytokines can significantly increase pro-inflammatory cytokines, such as the TNF-α, so cyclooxygenase (COX), lipoxygenase (LOX) enzymatic mediating mechanisms are triggered. The pro-inflammatory prostanglandins including 6-ketoprostaglandin F$_{1\alpha}$, thromboxane B$_2$, 8-isoprostaglandin F$_{2\alpha}$, and prostaglandins F$_2$, E$_2$, and D$_2$ can be used as biomarkers of the COX pathway during the inflammatory response in IBD. Meanwhile, biomarkers from LOX pathway include leukotrienes such as leukotriene B$_4$, C$_4$, D$_4$, and E$_4$ and hydroxyeicosatetraenoic acids (HETEs) such as 5-HETE and 15-HETE. Our goal was to develop a LC-MS method to monitor the change of these biomarkers in microdialysis sample to study the roles of LOX and COX enzyme activity in the inflammatory response in IBD. LC separation was obtained by a Phenomenex Kinetex XB-C18 column (100 mm ×2.1 mm i.d., 1.7 µm particle sizes) with binary solvent gradient elution. Solvent A consisted of water and 5 mM formic acid. Solvent B consisted of acetonitrile and 5 mM formic acid. The two-step linear gradient was: 0-18 minutes solvent B increased from 25 to 80%, and then remained at 80% for 7 minutes. MS results were acquired by a Thermo ESI ion-trap mass spectrometer. The microdialysis probe was implanted into the rat colon. Ringer’s solution was perfused to extract these biomarkers and then the dialysate was analyzed by the LC-MS method. All the 12 analytes were detected with good LOD, from 1-5 ng/L. The regression coefficients (R$^2$) from the calibration curve (1-50 ng/L) were over 0.999 for PGs and 0.98 for LTs and HETEs.
The cytoplasm is a dynamic environment that plays an important role in cellular metabolism. Sequential enzymes of several metabolic pathways are known to exist within close proximity, perhaps due to phase separation induced by macromolecular crowding within the cytoplasm. This has been described as the basis for microcompartmentalization and the subsequent colocalization of biomolecules may provide a means of regulation within a metabolic pathway. Aqueous solutions of structurally distinct polymers or a polymer and a salt phase separate over specific weight percentages. Enzymes and substrates preferentially partition to one of the phases, thus increasing local concentration while total volume remains constant. The common sequential enzyme pair of glucose oxidase and horseradish peroxidase was introduced into a polyethylene glycol (PEG):citrate biphasic system. Enzyme partitioning and product formation was monitored by fluorimetry while a mathematical model was used to elucidate the effects of compartmentalization by describing the reaction rates within each phase with respect to partitioning. Results indicate that the interface is influential for product formation when substrates and enzymes are localized in different phases and also suggest that complex reactions within heterogeneous media can be quantitatively described with such models.

**Keywords:** Bioanalytical, Biospectroscopy, Biotechnology, Fluorescence

**Application Code:** Bioanalytical

**Methodology Code:** Biospectroscopy
The developments and wide applications of nanomaterials in the past decades have increased dramatically, drawing people's attention to their potential adverse impacts on the environment and human health. Titanium dioxide (TiO2) nanoparticles (NPs) are manufactured worldwide and widely used in many fields. Although many studies have demonstrated the cytotoxicity of TiO2 NPs, the mechanism is not well understood and needs to be further investigated. It is anticipated that when cells are exposed to external stimuli, such as high concentrations of NPs, the changes of cellular activities, such as differentiation, gene expression, metabolism, and others, may alter the intercellular and extracellular pHs. Due to the heterogeneous characters of cells, the pH change among different cells maybe different upon exposure to the same concentration of TiO2 NPs. Therefore, monitoring pH at single cell level is utmost important to understand the response of each individual cell to the change of the environment. In this study, a newly developed micro-pH probe has been applied to measure the pH profile of cell surface at single-cell level. The effects of different sizes of TiO2 NPs on pH changes at cell surface with different time domain and space domain were investigated. Results showed that pH of cell surface decreases continuously after dosed with TiO2 NPs and the decreasing rate of pH of cells correlates well with the distance between cells and the TiO2 NPs. The detail experimental conditions, results, and discussions will be presented at the conference.

This research is supported by National Institutes of Health (NIH).
The organic ligands surrounding metal nanoparticles (NPs) not only provide stability and control growing during the synthesis but also, create a flexible platform for sensing volatile organic compounds (VOCs). In this work we chemically synthesized surfactant (tetraoctylammonium bromide)-coated Au NPs and assemble them as films for the detection of VOCs via distinct changes in plasmon band, interparticle distance, and film structure. For instance, changes in distance between NPs upon VOCs have been usually speculated on the basis of indirect results such as changes in film current or plasmon band. Here, we performed in-situ GISAXS and SAXS experiments in order to unambiguously monitor interparticle distance and film structure upon measured concentrations of polar (ethanol) and non-polar (toluene) vapors. Results show distinct film behavior depending on both; the nature of the analyte vapor and organic alkyl chain. In this presentation I will discuss the importance of film flexibility provided by the organic matrix during VOCs sensing. I will show how the organic alkyl chains are responsible of film restructuring, changes in distance between NPs, and distinct plasmon shift towards red and blue depending on the polarity of the vapor analyte.

Keywords: Headspace, Nanotechnology, Sensors, Volatile Organic Compounds
Application Code: Nanotechnology
Methodology Code: Sensors
A facile approach for shape-controlled synthesis of gold nanoparticles (Au NPs) using 5-hydroxyindoleacetic acid (5-HIAA) as the reducing agent has been reported. Au NPs of various shapes (triangles, hexagons, and semi-spheres, etc.) were prepared by varying the ratio of chlorauric acid and 5-HIAA, temperature, and reaction time. A detailed characterization was performed using ultraviolet-visible (UV-Vis) spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy. The as-prepared Au NPs exhibited excellent surface-enhanced Raman scattering (SERS) properties, which make them extremely attractive for the development of SERS-based chemical and biological sensors.
Predictable performance of immobilized proteins on many surfaces is a required condition to functional scaffold materials for potential applications in biosensing, tissue engineering, and proteomic platforms. We have utilized polydiacetylene liposomes as an immobilization scaffold for evaluating the behavior of substrate-enzyme reaction activities. Liposomes composed of polymerized 10,12-pentacosadiynoic acid and dimyristoylphosphatidylcholine were used for immobilization of enzyme and substrates at the bilayer. The resultant protein or enzyme covalently bound to liposomes interacted with free Trypsin (Rh-TRY) or fluorescein-BSA (Fl-BSA) forming Liposome-Protein-Enzyme (BiPE) or Liposome-Enzyme-Protein (BiEP) complexes respectively. The subtle biological interactions occurring at the bilayer surface were monitored by using electronic absorption spectroscopy, fluorescence anisotropy (FA), and spectral imaging. Both colorimetric response (CR) and FA confirmed enzymatic reaction for BiEP and BiPE. CR increased to 35% and FA decreased from 0.29±0.001 to 0.04 suggesting enzymatic cleavage of bound protein into bulk solution. The fluorescence imaging confirmed the enzymatic cleavage of BSA which showed the fluorescein emission intensity decreased ~60% after one hour of enzymatic reaction. In the second case, BiE generated CR 8% which increased to ~25% after the addition of free Fl-BSA probably due to stress imposed by enzyme-protein interactions at the conjugated bilayer. It was discovered that BiPE imposed a much larger stress on the bilayer compared to BiEP. Ultimately, we show that the interactions between enzyme and substrate at the bilayer can provide useful information, and it acts as a model system for potential creation of novel technologies as promising biomolecules-liposomes hierarchal complex structure.
Preparation and Characterization of Nanomaterial Biosensors

Electronic Interaction Between Molecular Machines and Plasmonic Nanoantennas

The photo-reversibility of molecular machine-attached onto anisotropic nanostructures have been studied using optical spectroscopy. For the first time, we have observed an unprecedented 21-nm shift of localized surface plasmon resonance (LSPR) peak of gold nanoprism upon cis to trans isomerization of azobenzenes. The observed shift was a combined effect of energy transfer across the nanostructure and azobenzene molecule and increased in the dielectric environment of the nanostructure. We also investigate the mechanism underline large LSPR peak shifts by fine-tuning the distance between azobenzene and gold nanoprism. Understanding such mechanism will aid in designing of highly efficient sensing platforms for future optoelectronic device fabrication.

Keywords: Nanotechnology, Spectroscopy, Surface Enhanced Raman
Application Code: Nanotechnology
Methodology Code: Molecular Spectroscopy
In this work, glassy carbon electrodes (GCE) modified with Self-Assembled (SA) films of polyaniline (PAni) and carboxylic acid functionalized multiwalled carbon nanotubes (MWCNT-COOH) were developed and applied to determine the pesticide 2,4-D. Three different SA films were produced, with 20 alternated layers of PAni or its composite with 5 percent in mass of MWCNT (PAni 5% MWCNT) and with an alternated layer of Polystyrene Sulphonated (PSS). The scanning electron microscopy results (MEV) showed the presence of MWNT that was more evidenced into the PAni/MWCNT/PAni/PSS film. The cyclic voltammetry (CV) results showed an increase of the peak current characteristic of the conducting polymer (PAni) with the increase of the MWCNT proportion. The PAni/PSS film was able to detect 2,4-D by the increase of the current signals even in aqueous media with pH 7.0.

Keywords: Electrochemistry, Electrode Surfaces, Nanotechnology, Voltammetry
Application Code: Nanotechnology
Methodology Code: Electrochemistry
Monolithic nanoporous gold nanoparticles are of great interest due to the unique 3-dimensional bicontinuous nanostructures with large surface area, tunable plasmonic resonance and high-density hot-spots. Recently we have also demonstrated their potential applications in surface enhanced spectroscopy, photothermal conversion and plasmonic sensing. In existing applications, nanoporous gold nanoparticles have been employed as dense particle arrays fixed on glass or Si substrates. In other words, little has been done in developing colloidal nanoporous gold nanoparticles as a platform to form various nanocomposites with other nanomaterials. In this presentation, we show that by tuning the pore size, small gold nanoparticles can be loaded into the porous structure or attached at the surface of nanoporous gold nanoparticles to form nanoporous gold nanocomposites. The strong plasmonic coupling not only originates from the interaction between nanoporous gold nanoparticles and small gold nanoparticles, but also between small gold nanoparticles. The plasmonic resonance can be easily tuned in both visible and NIR spectral ranges by changing the diameter of attached gold nanoparticle sizes. In addition, potential applications of nanocomposites in surface-enhanced Raman scattering and plasmonic sensing will be discussed.
Resistive pulse sensing with two nanopores in series is used to measure size and mobility of individual hepatitis B virus (HBV) particles as they self-assemble in real time. A nanochannel with two nanopores in series is milled in glass by a focused ion beam to bridge two V-shaped microchannels. The assembly product is driven through the nanochannel by application of a positive potential, and two decreases in ion current are measured for each transiting species. HBV forms two icosahedral capsids, a 90-dimer capsid with T = 3 symmetry (32-nm diameter) and a 120-dimer capsid with T = 4 symmetry (35-nm diameter), which are discriminated by their relative ion current displacement. Current displacement is proportional to capsid volume, and the 3-nm difference in outer diameter between T = 3 and T = 4 capsids is easily resolved. Capsid velocity is determined from the transit time between pore one and pore two and is used to calculate electrophoretic mobility. Virus assembly is initiated by mixing HBV dimer with a high salt buffer, and assembly is monitored over minutes to hours. Under normal reaction conditions, T = 4 capsids are the dominant product (90%) and resistive-pulse sensing easily differentiates between a T = 4 capsid and a T = 3 capsid, which often unresolved by conventional measurement techniques such as light scattering. Our single-particle counting method reveals differences in the assembly kinetics of T = 3 and T = 4 capsids.
The use of supercritical CO2 mobile phases or convergence chromatography (CC) is an attractive alternative to liquid chromatography that provides different selectivity, low solvent usage, and high efficiency separations. To fully take advantage of the potential efficiency gains, greater linear velocities are desired. Furthermore, the analysis of polar analytes by supercritical fluid chromatography (SFC), a normal phase technique, can require a high percent of organic co-solvent for elution. Therefore, the analysis of polar analytes by SFC requires robust instrumentation that can accommodate high flow rates and high solvent viscosities.

To fully realize the benefits of convergence chromatography, it is important to screen a wide range of variables during method development, including organic co-solvent composition, column temperature, column dimension, flow rate, and applied back-pressure. Each of these variables impacts not only the chromatographic separation, but also the overall speed and efficiency of analysis. Increasing the linear velocity to aid in throughput will also increase the overall system pressure. This phenomenon is especially noticeable for SFC methods requiring a higher percentage of organic co-solvent and is compounded further if a complex separation requires the use of longer columns which also generate an increase in system pressure.

Combining convergence chromatography, with instrumentation that has an elevated allowable system pressure, we were able to test a wider range of parameters during method development, ultimately leading to faster, higher efficiency separation methods. Specifically, polar compounds which require high amounts of organic co-solvent for column elution were now run with increased flow rates resulting in more efficient separations and higher sample throughput.

**Keywords:** Analysis, Mass Spectrometry, Method Development, Supercritical Fluid Chromatography

**Application Code:** Pharmaceutical

**Methodology Code:** Supercritical Fluid Chromatography
Session Title: Supercritical Fluid Chromatography

Abstract Title: Improving Efficiency in a Quality Control Laboratory by Leveraging the Recent Advances in Supercritical Fluid Chromatography

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Abstract Text:

Quality Control (QC) Laboratories are continually looking for ways to improve efficiency and reduce sample turn-around-time. With this goal in mind the latest advances in Supercritical Fluid Chromatography (SFC) were leveraged for the separation and quantitation of active ingredients in Health, Beauty and Nutrition Products that were previously determined by HPLC and UPLC methods. Method Equivalency was demonstrated by comparing results obtained for 6 sample preparations analyzed by each technique through statistical analysis via a two, one-sided t-test (TOST) at the 95% confidence interval. Nine QC methods were converted that resulted in several efficiency benefits: instrument run time was reduced by 79%, the number of mobile phase solvents was reduced by 82%, the volume of mobile phase required was reduced by 95% and the number of analytical columns was reduced by 78%. Additional benefits were realized by utilizing the column manager and pump module which removed the need to manually switch columns and solvents between analytical runs allowing for the automated sequential testing of different sample sets. Based on the results obtained from this evaluation the recent advances in SFC make it a competitive choice to HPLC and UPLC methodology.

Keywords: Analysis, Chromatography, Consumer Products, Supercritical Fluid Chromatography

Application Code: Consumer Products

Methodology Code: Supercritical Fluid Chromatography
Supercritical Fluid Chromatography

Determination of Brominated Vegetable Oil in Soft Drinks by UPC2-MS

Brominated vegetable oil (BVO) is often used as a weighting agent, or a solubility-transmitter for citrus oils and other lipophilic compounds1 in soft drinks and beverages. The US FDA has established a BVO limit at 15 ppm in finished beverages, while many countries in Europe, Asia, South America, and Australia, have banned its use in beverages. Analysis of BVO is rarely reported. Gas chromatography with mass spectrometry (GC-MS) has been proposed recently for the analysis of BVO in soft drinks and cocktail syrups2,3. This GC-MS method requires tedious derivatization (or saponification) of BVO, and has a long run time (about 50min). UltraPerformance Convergence Chromatography™ (UPC2®) is a state-of-art supercritical fluid chromatography (SFC) that provides exceptional efficiency and speed of separation4. It has been applied to a wide range of compounds, including VO, and has shown great benefits in selectivity, throughput, and ease-of-use5. This work demonstrates a rapid and simple analysis of BVO in soft drinks and beverages using UPC2-MS. BVO was extracted and analyzed directly without any derivatization. The chromatography total run time was 9 min. The analytical method performance (limit of quantitation or LOQ, repeatability, linearity, and recovery) as well as the analysis of BVO in soft drinks and beverages are presented.

Keywords: Food Contaminants, Mass Spectrometry, Quality Control, Supercritical Fluid Chromatography
Application Code: Quality/QA/QC
Methodology Code: Supercritical Fluid Chromatography
Advancements in SFC instrumentation with specifically designed column chemistries have provided a more robust chromatographic solution. With the new technology, we have also improved the traditional SFC method development strategy, which often took a brute force screening approach. Extensive libraries of column chemistries and multiple combinations of modifiers and additives are no longer necessary to develop successful SFC separations. Using novel, sub-2 µm UPC² achiral chemistries coupled with ACQUITY UPC² instrumentation, we have created a simplified method development strategy by implementing a single column scouting run. Resulting selectivity and peak shape from our 2-Pic chemistry will then direct the user to further optimize or select another specific chemistry and/or specific co-solvent to meet the separation criteria. Research used to design the strategy shows which methanol based co-solvents provide the largest impact on each new chemistry and how chemistry/co-solvent combinations compare to each other. ACQUITY UPC² technology provides a complete system platform that allows users to quickly develop robust methods by accessing a wide selectivity space with exceptional peak shape and separation performance.

Keywords: Chromatography, Method Development, SFC
Application Code: Other
Methodology Code: Supercritical Fluid Chromatography
Bioanalysis and drug metabolism studies are critical parts of the drug development process. The aim of these studies is to identify and quantify drugs and their associated metabolites in biofluids such as plasma and urine. Typically reversed-phase chromatography coupled with mass spectrometry is often the analytical technique of choice utilized in the analyses due to the specificity and sensitivity of the technique. However, due to the complexity of the biofluid samples accurate and precise measurements can become challenging due to poor chromatographic peak shape, insufficient chromatographic resolution from matrix components and incompatible sample compositions.

Recent advancements in the field of supercritical fluid chromatography (SFC) have lead to the development of sub 2 micron chromatographic separations coupled mass spectrometry operating under positive ESI mode. In the work presented here the applicability of SFC for both chiral and achiral bioanalysis is shown. The orthogonal separation selectivity compared with reversed-phase separations and tolerance for high organic sample compositions will be discussed. This work further presents a critical evaluation of the influence of mobile phase and make up flow modifiers on the sensitivity and selectivity of probe pharmaceuticals analyzed under SFC/ESI positive mode MS conditions.

Keywords: Bioanalytical, Biological Samples, Chromatography, Separation Sciences
Application Code: Bioanalytical
Methodology Code: Supercritical Fluid Chromatography
Separations via supercritical fluid chromatography (SFC) continue to proliferate as an environmentally friendly separation technique, particularly in format similar to that of packed column liquid chromatography (LC). Stationary phases typically used in HPLC have been explored in SFC. Amide-based columns, where a polar group is embedded in the hydrophobic backbone of a reversed phase type material, provide alternate selectivity towards more polar compounds. These types of phases have not been studied much under SFC conditions. This study has the objective evaluating a C18-amide stationary phase using SFC. The amide column is compared with bare silica and C18 phases. The system was held at supercritical pressure and temperature with a mobile phase composition of carbon dioxide and a co-solvent. The linear solvation energy relationship (LSER) model was used in an attempt to understand the behavior of these materials, relating the retention factor of selected probes to specific chromatographic interactions. Preliminary results show that the C18-amide stationary phase posses combine interactions, as one would expect. The hydrophobic interactions are favorable; however, the electron donating ability of the amide group also shows a large positive interaction. Details of the chromatographic evaluation will be discussed.
Countless lives have been saved due to widespread blood banking efforts since the advent of blood transfusions and introduction of effective storage conditions. However, managing the supplies of stored blood products continues to be an administrative challenge. Presently, stored red blood cells (RBCs) are considered to be viable for 42 days, but there is strong evidence that blood from different donors does not degrade at the same rate. Observational studies have suggested that these variations are responsible for a variety of post-transfusion illnesses. It would therefore be advantageous to assess the condition of stored blood rapidly and accurately without needing to sample the contents of the bag. When stored in a plastic blood bag, RBCs are known to undergo a multitude of chemical, physiological, and morphological changes over time. Many of these age-related changes (for example, loss of hemoglobin or a decrease in metabolic regulators) produce chemical species that can be characterized using Raman spectroscopy. This study clearly demonstrates that the Raman spectrum of stored RBCs changes as a function of storage age, most notably with specific bands related to the oxygenation state of hemoglobin and supernatant spectra that offers insight into small molecule externalization. Chemometric methods reveal the biochemical components that have previously been associated with ageing. The presented data will show that Raman spectroscopy has promise as a tool for monitoring RBC storage.
Soft tissue injuries are painful, disabling, and costly. While actual tendon and ligament ruptures are rare, sub-rupture strains and sprains present substantial clinical problems. We have provided the first evidence that overload failure of soft tissues such as tendons is governed by a novel mechanism: discrete plastic failure of collagen fibrils in a series of kink-like structures that develop along the fibril's length. FTIR spectrochemical imaging is one of several techniques that we use to characterize normal, stressed and overloaded tendon. A recent optics upgrade to our thermal source FTIR microscope (Agilent Cary 670/620, 64×64 Focal Plane Array detector) enabled rapid acquisition of spectrochemical images with an effective geometric pixel size (~1 micon edge) at the sample plane. Rapid ex vivo tissue imaging is done in-house, with images collected over larger areas, in less time (minutes), with comparable quality and resolution to the best synchrotron source FTIR imaging capabilities. Image files, initially processed with the Agilent ResPro software, are exported to MatlabTM where the distribution of integrated absorbance peak values is modelled using a Gaussian mixture with two components. The model components for each peak are used to classify each spectrum into the model-appropriate groups. Spectra with alike classifications are averaged to identify differences within the sample. Matched-pair samples are assessed spectroscopically (FTIR) and visually (SEM), so that overload-induced differences in spectra can be directly related to alterations in the tendons' collagen fibril nano-architecture. We will present analysis and implications from our first results on normal and overloaded tendons, prepared with and without prior decellularization.
Gold microhole arrays are a highly sensitive plasmonic substrate, which can be easily produced by either colloidal lithography or photolithography. We previously reported improved sensitivity by three fold for an IgG bioassay using SPR in Kretschmann configuration and microhole arrays. The micrometric features of microhole arrays lead to the excitation of propagative and localized surface plasmons. In this work, the localized electromagnetic field is exploited for metal enhanced fluorescence (MEF). The design of a SPR-fluorimeter allowed the simultaneous detection of both SPR and fluorescence signals in a labelled protein bioassay. A simultaneous detection of correlated fluorescence and SPR signals will lead to more reliable and sensitive immunoassays. We investigated the MEF phenomenon and optimized the microhole arrays parameters by changing both thickness and hole diameter to obtain maximal enhancement in both SPR and fluorescence signals. Up to three-fold fluorescence signal enhancement was obtained for rhodamine 6G deposited on microhole arrays, in comparison to standard thin gold films. As a proof of concept, we used a PSA sandwich-immunoassay and were able to detect both SPR and fluorescence signal. Thereby, we envision bioanalysis using an hour-long procedure on an instrument the size of a lunch box.

Keywords: Biosensors, Fluorescence, Nanotechnology, Sensors
Application Code: Biomedical
Methodology Code: Fluorescence/Luminescence
The degradation of carbofuran, a frequently carbamate derivative pesticide that is considered a priority pollutant, was investigated. This work has been conducted to study the factors (temperature, UV-rays, direct sunlight, and pH) affecting the biodegradation of carbofuran pesticide by irradiated fungi. The residue of carbofuran was analyzed by high-performance liquid chromatography (HPLC). The results showed that calculated half-life times of carbofuran were 121.95, 102.87, and 36.00 hours at 25, 35, and 45 ºC, respectively, when exposed to different temperatures. The statistically determined half-life times for carbofuran were 15.14 hours after exposure to UV-rays as a thin film in glass surfaces. The results clearly showed that photodecomposition of carbofuran occurred after one hour of exposure to sunlight. The percent loss for carbofuran was 78.70% after one hour of exposure to sunlight. The results also demonstrated that no carbofuran was detected after 4 days from exposure to pH 9 at 45 ºC.
Batteries and cell phone batteries cause a serious environmental problem today, by presenting in its Constitution and health harmful metals to the environment such as mercury, lead, copper, zinc, cadmium, manganese, nickel and lithium. Classified as class I, hazardous waste, once used, most batteries are thrown in common waste. Studied matrices are landfill waste, garbage, compost manure and sewage sludge. The mud did not show toxic metal concentrations below those required by Brazilian legislation for the use of agricultural waste. The residue of landfill garbage compound showed high levels of Zn, Pb, Cd, Ni, Cr, Mn, Cu and the toxic metals that contaminate the soil, water courses and water table, reach the flora and fauna of the surrounding regions. Through the food chain, these substances come to humans, may cause intoxications, depending on the dose absorbed.
Nitrogen and Carbon Determination in Soils and Plants by Flash Combustion Using Argon as Carrier Gas

Nitrogen in soils is important for the evaluation of organic matter and the calculation of the amount of fertilizer to be added by providing information regarding the deficiency or excess of nutritional elements important for plants growth. Nitrogen content is important in determining the quality of various types of crops for feeding and processing, as well as for N-cycle and N-fixation monitoring in agricultural and environmental research. Nitrogen and Carbon analysis in soils gives information pertaining to the deficiency or excess of nutritional elements. For this reason the use of an accurate instrumental analytical techniques is required. As the demand for improved sample throughput, reduction of operational costs and minimization of human errors is becoming every day more notable, it is very important apply a simple and automatic technique which allows the fast analysis with excellent reproducibility. The FLASH 2000 Analyzer, using typically Helium gas carrier and based on the dynamic flash combustion of the sample, copes effortlessly with the wide array of laboratory requirements such as accuracy, day to day reproducibility and high sample throughput. However as in the last years there is a possible worldwide shortage and large cost increasing for Helium, it is necessary to test as alternative gas, Argon which is readily available. This paper presents data on Nitrogen and Carbon determination in soils and plants reference materials with different Nitrogen and Carbon concentration to show the performance of the system using Argon as carrier gas and the reproducibility of the results obtained.

Keywords: Agricultural, Elemental Analysis, Soil
Application Code: Agriculture
Methodology Code: Other
Se deficiency in dairy cows can cause serious problems such as retained placentas at calving and high somatic cell counts in milk, which in turn reduces milk quality and quantity and increases the risk of mastitis. Se deficiencies in newly born calves and lambs can result in ill-thrift, brought about by reduced eagerness to suckle milk and white muscle disease. It is therefore common place to supplement for these deficiencies with inorganic minerals such as sodium selenite. However, ruminants are less able to absorb inorganic Se (<50% absorbed) compared with organic forms. In addition, the inorganic form of Se is less readily converted into milk Se than its organic counterpart. It is therefore important to be able to determine the Se status of ruminant feeds.

It is a challenging task to quantitatively analyse selenium species in livestock feed. Five selenium species have been investigated in this study. The extraction procedure from livestock feed samples and the separation of the five selenium species is investigated and optimised. HG-AFS technical is used for the detection of Se signals with detection limits between 0.13 and 0.43 ng (3s).
In the past, there have been several major dioxin and polychlorinated biphenyl (PCB) contamination incidents in food and feed industries such as the Belgium crisis in 1999. This event and other major accidents in Europe demonstrated the importance of monitoring/removing dioxins/PCBs in food and feed. Currently, activated carbon (AC) is the most widely used agent to adsorb dioxins and PCBs in the feed industry. However, the main mechanism for AC adsorption is the planarity effect. In other words, non-dioxin like PCBs (the two phenyl rings of the PCB congeners are not coplanar) cannot be effectively removed by AC (<30% removal). In addition, due to the limited selectivity, AC treatment typically requires elevated temperature (70-80 oC) and special conditions like low pressure (50 mbar) or countercurrent supercritical CO2 extraction.

Here, a molecularly imprinted polymer (MIP) adsorbent for dioxins/PCB was prepared using a non-covalent fragment imprinting technique. MIP particles were synthesized in methylene chloride/methanol mixture by using 1,2,3,4,5-pentachlorobenzene (PenCB) as a template, 2,4,6-trimethylstyrene (TMS) as the functional monomer, and ethylene glycol dimethacrylate (EGDMA) as the cross-linker. The MIP showed an excellent affinity towards dioxins and PCBs (including non-dioxin like PCBs) in aqueous solution, and the adsorption efficiency is 1.2 ± 0.3 mg kg⁻¹. All dioxin and PCB congeners (10 ng) were nearly completely removed (less than our detection limit) by 3 mg MIP treatment within 30 minutes at room temperature.

Keywords: Adsorption, GC-MS, PCB's
Application Code: Agriculture
Methodology Code: Gas Chromatography/Mass Spectrometry
Metabolomics provides an alternative to supplement compositional analysis for substantial equivalence assessment of genetically modified crops. Therefore, understanding of biological variation in metabolites due to multiple factors such as environment (growing location), genetic modification, planting season is of great importance. In this study, two common maize sample sets from the year of 2012 (180 samples) and 2014 (90 samples) were selected, including 5 genetically diverse non-GMO DuPont Pioneer commercial maize hybrids grown at six North America locations. GC/MS and LC/MS were used as complementary analytical platforms to detect a wide range of compound classes. Genedata software was used for data pre-processing and integration. A total of 157 and 139 metabolites were detected in forage and grain samples by GC/MS, while 657 and 256 metabolites were detected in forage and grain samples by using LC/MS positive mode and negative mode combined. Univariate and multivariate statistical analyses were utilized to evaluate the main effects and interaction of environment, genotype, and planting season on the maize metabolome. The results showed that the environment had far more impact than genetic background, which were consistent with previous findings. Moreover, seasonal effects within each location were greater than genotype effects. 30% to 63% of the metabolites in forage samples, and 19% to 65% of those in grain samples, were significantly altered between the two seasons, compared to less than 10% of the metabolites affected by genetic modifications.
Acid-base equilibrium is related to almost all process occurring in soil. The bioavailability of nutrients for plants, for instance, depends on the solubilization of mineral nutrients in the soil solution, a pH-dependent process. The determination of pH in soil solutions is usually carried on by potentiometric measurements. The soil solutions are prepared from extractions of some soil components with water or CaCl₂ solution. In this proposal a new, simple and direct method for soil pH determinations is proposed using laser induced breakdown spectroscopy (LIBS). Sixty samples presenting different textural composition and having their pH previously determined by pH electrode were considered. LIBS spectra were recorded for each sample in pellets format, using a laser pulse energy of 115 mJ. The laser operated at 532 nm, 4.4 ns pulse duration, and 10 Hz repetition frequency. Intensities of thirty-three emission lines from LIBS spectra, corresponding to emission of Al, Ca, H and O, were used to delineate a partial least square model, which was validated using ten folds cross-validation. The pH values predicted by the proposed model showed a strong correlation with reference values (R= 0.83) and a prediction mean absolute error of 0.3 units of pH. These results highlight the LIBS potential for other determinations beyond the sample elemental composition. Considering soil analysis, the proposed method encloses the possibility of pH determination in addition of nutrients and contaminants using a single LIBS measurement.

Acknowledgements: The authors thanks to FAPESP and Fundación Carolina and Jesús Anzano thanks to European Social Found, Government of Aragón and the University of Zaragoza (proposal #E75).
A suitable reverse phase HPLC (RP-HPLC) analytical method was implemented for the quantitative extraction and determination of “active ingredient A” in experimental samples from a very complex soil matrix. During the greenhouse study, soil samples were collected at different time points, expressed in days.

The RP-HPLC separation was carried out by reverse phase chromatography on Zorbax Eclipse C8 column (3.0 x 150 mm, 3.5[μ]m); a gradient run using mobile phase A: MilliQ water + 0.01% formic acid, mobile phase B: acetonitrile + 0.01% formic acid; flow rate = 0.5 mL/min; injection volume 5 [μ]L and UV-VIS detection at 260nm. The accuracy was calculated, the percent recovery was 98-101% and reproducibility was found to be satisfactory. The calibration curve for “active ingredient A” was linear from 2.5 to 250 [μ]g/mL, where the limit of detection and limit of quantification were 1 and 2.5 [μ]g/mL, respectively. The inter-day and intraday precision was found to be within limits. The present analytical method is accurate, precise and it was successfully applied to analyze these research samples.
Gamma-aminobutyric acid (GABA), four carbon non-protein amino acid, is proposed to be a signaling molecule in response to various abiotic stresses in plants. Salinity and drought are important environmental stress factors that negatively affect plant survival and productivity. The aim of this study was to evaluate the effects of drought and salinity on GABA in Phaseolus vulgaris L. plants. GABA content was determined by HPLC (Agilent 1200) with a modified method. Pre-column derivatized samples were separated with RP-HPLC on a C18 column and UV detection (330 nm) with flow rate 1 mL min⁻¹ and 5 µL injection volume. High salinity increased GABA levels compared with drought stress conditions. However GABA content was decreased with the effect of combined stress conditions. According to our results we can suggest that GABA metabolism is strongly related with primary metabolism products, also drought and salinity stress conditions. We further evaluate the effects of different environmental stress factors on GABA metabolism in different plants.
## Abstract Text

Cell surface proteomics has seen momentous developments in the past two decades but still faces major challenges in location verification of identified cell surface proteins (CSPs). Recent approaches focus on modification/labeling of CSPs by chemical reagents followed by mass spectrometric analysis of labeled CSPs. Popular biotinylation regents have shown some intrinsic disadvantages such as internalization in the cell cytoplasm, poor recovery of biotinylated proteins, presence of endogenous biotin and non-specific interactions between avidin and proteins. In our study, silica coated iron oxide (Fe3O4@SiO2) superparamagnetic nanoparticles (MNPs) of 100~150 nm were utilized to prepare an impermeable and magnetically separable cell surface labeling reagent. Sulfo-N-hydrosuccinimidyl (NHS) ester group was conjugated to the surface of Fe3O4@SiO2 MNPs via a disulfide bond to facilitate removal of the magnetic nanoparticle moiety after separation. The surface exposed amine groups of *Saccharomyces cerevisae* were modified at physiological pH on ice to preserve the native structure of CSPs. Electron microscopic analysis of MNPs conjugated to the *S. cerevisae* cell surface confirmed the impermeable nature of sulfo-NHS ester Fe3O4@SiO2 MNPs. The labeled CSPs were easily separated by using a magnet and eluted from MNPs by cleaving a disulfide bond. The LC-MS/MS analysis of labeled peptides revealed 30 surface proteins located on solvent exposed surface of the *S. cerevisae*. The sulfo-NHS ester modified Fe3O4@SiO2 MNPs offers benefits such as impermeability, quick magnetic separation of labeled peptides and labeling under physiological conditions.

**Keywords:** Mass Spectrometry, Nanotechnology, Proteomics, Tandem Mass Spec

**Application Code:** Genomics, Proteomics and Other `Omics

**Methodology Code:** Mass Spectrometry
Magnesium and its alloys are receiving increased interest in the field of biodegradable materials because of their good metallic strength, light weight, low toxicity and rapid corrosion rate in aqueous solutions. Various magnesium alloys have been developed in order to better control their corrosion rate, but due to the lack of standard procedures in testing the corrosion rate in vivo and in vitro, few systematic studies have been reported. We have developed the capability for monitoring in real time the Mg corrosion process in vivo for corrosion products using a H2 sensor. We also established a protocol for in vivo corrosion studies to obtain the surface composition of explanted Mg alloy by surface spectroscopies such as XPS; the distribution of corrosion products in the tissue surrounding an implant and in critical organs of test animals using bioanalytical techniques involving extraction and ICP-MS.
Volatile nitrosothiols (vSNOs) are presented as a compound class that should be taken into consideration for cancer disease markers in breath and from tissue emissions. Methyl-, ethyl-, 2-propyl- and allyl-SNOs have been synthesized in a two phases reaction between an aqueous layer covered with n-decane. The reaction is carried out with the corresponding thiols and sodium nitrite in the presence of oxalic acid. The reaction solution turns into red due to the production of the colored vSNOs. The evolving and decomposition of the vSNOs in the reactor system has been monitored by online-MS/MS of the headspace gas of the reaction solution. Spectra of the vSNOs among educts and decomposition products have been recorded by loop injection on fast GC/MS at sub ambient temperatures. Storing the reaction mixtures as diluted n-decane solutions in air tight diffusion devices allows handling them for weeks without decomposition and to train sniffer dogs. The behavior of sniffer dogs has shown that the proposed vSNOs seem to be very similar or even identical to cancer odor emission. Details of the synthesis, pictures of the reaction solutions, analytical runs and construction parts of the diffusions devices are presented.
Interaction of Cancer Cells with Microposts in a Microfluidic Device Immobilized with Aptamers

Circulating tumor cells (CTCs) show great potential for cancer diagnosis as well as guiding cancer treatment. However, the rare number of CTCs in peripheral blood hinder its application, making its detection and analysis a big challenge. Antibody-based CTC isolation method has been proved to be one of the most efficient method for CTC enrichment in patient blood. Microfluidic device with micropost array in microchannels, a popular device used for antibody-based CTC isolation, gives high CTC capture efficiency and purity due to its specific geometry. However, how CTCs interact with such a microfluidic device under different flow conditions remain unclear. We report here cancer cell distribution around microposts under different flow conditions. Homogenous human acute lymphoblastic leukemia cells (CCRF-CEM) were used as target cells in this study. We used high affinity aptamers as an alternative to antibodies for cell capture. Flow rates and post shapes were studied as the parameters that affect flow condition. While higher flow rate decreased cell capture efficiency, cell captured locations around microposts also changed, leading to higher ratio between cells captured around the front half of a micropost vs cells captured around the back half of a micropost. As comparison with experimental result, simulation based on COMSOL was conducted. A 2D model was set up to model a section of a microchannel with a micropost array. The detailed results and comparison will be presented in this paper.

Keywords: Biomedical, Lab-on-a-Chip/Microfluidics
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
## Abstract Text

Synthetic oligonucleotides are typically purified using ion-pair reversed-phase HPLC. One problem for preparative scale separations has been the restriction of mass transfer within the porous network or exclusion of larger molecules from the pores altogether. Use of elevated temperatures (> 60 [degree]C) improves mass transfer, leading to improved resolution and peak shape, however, silica-based C18 (ODS) columns have diminished lifetimes under such conditions. Instead, columns based on poly(styrene-divinylbenzene) (PS-DVB) have been attractive alternatives due to their superior thermal (>100 [degree]C) and pH stability (pH 1-13). However, predecessor PS-DVB beads suffer from poor mechanical stability and lower efficiency compared to ODS.

In this study, a porous, mechanically-stable (> 5,000 psi) C18-functionalized PS-DVB-based column has been evaluated for analysis and preparative scale purifications of trityl-off DNA oligonucleotides. Resolution and loading for the C18-alkylated PS-DVB column was superior to both unmodified PS-DVB and organic/inorganic hybrid silica-based C18. The thermal stability of the new polymeric support permits use of elevated temperatures (up to 100 [degree]C) to further enhance efficiency. Long (> 60mer) DNA oligonucleotides could be separated from n-1 truncated sequences in 10 minutes with single nucleotide resolution.

### Keywords:
- Gene Therapy
- HPLC Columns
- Nucleic Acids
- Prep Chromatography

### Application Code:
- Biomedical

### Methodology Code:
- Liquid Chromatography
Detection of Doping Agents in Serum Using a NanoSPRi Platform

Misuse of growth hormones and steroids have become a recurring problem amongst professional and amateur athletes. Not only performance-enhancing drugs undermine ethical issues in competitive sports but also present serious health threats to the abuser. Recombinant human growth hormone (rhGH), a 22 KDa polypeptide, is a banned substance by the World Anti-Doping Agency and much effort by international researchers has been focused onto developing tests that can reveal its presence or anabolic effect. A number of detection tools such as mass spectroscopy (MS) and enzyme-linked immunosorbent assay (ELISA) have been developed to monitor the level of these drugs in blood as a reliable measure of doping; however, these tests suffer from either lack of sensitivity or laborious work. In this study, the diagnostic potential of a Surface Plasmon Resonance imaging (SPRi) in combination with semiconducting nanoparticles (NanoSPRi) or without was assessed for measuring hGH in serum and was compared with commercially available ELISA kits. Our results strongly suggest that SPRi and Nano-SPRi encompass many advantages over ELISA.

Abstract Text

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Abstract Title

Biomedical Applications

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Abstract Text

Misuse of growth hormones and steroids have become a recurring problem amongst professional and amateur athletes. Not only performance-enhancing drugs undermine ethical issues in competitive sports but also present serious health threats to the abuser. Recombinant human growth hormone (rhGH), a 22 KDa polypeptide, is a banned substance by the World Anti-Doping Agency and much effort by international researchers has been focused onto developing tests that can reveal its presence or anabolic effect. A number of detection tools such as mass spectroscopy (MS) and enzyme-linked immunosorbent assay (ELISA) have been developed to monitor the level of these drugs in blood as a reliable measure of doping; however, these tests suffer from either lack of sensitivity or laborious work. In this study, the diagnostic potential of a Surface Plasmon Resonance imaging (SPRi) in combination with semiconducting nanoparticles (NanoSPRi) or without was assessed for measuring hGH in serum and was compared with commercially available ELISA kits. Our results strongly suggest that SPRi and Nano-SPRi encompass many advantages over ELISA.

Keywords:

Biopharmaceutical, Biosensors, Biotechnology, Nanotechnology

Application Code:

Biomedical

Methodology Code:

Biospectroscopy
Proteins exist in a highly ordered, folded state. This highly ordered structure of a protein is integral to the efficacy and safety of the protein-based bio therapeutic. Conformational changes of proteins by solvent induced unfolding may result in conformational changes, even if not completely denatured. As a protein begins to unfold, the hydrodynamic radii of protein species change. Different proteins are susceptible to solvent induced unfolding to a different extent. The change in the conformation due to solvent induced unfolding is possible to be identified by the associated volume changes by size-exclusion chromatography and can be detected by the change in the intrinsic fluorescence of the tryptophan containing proteins. Larger the molecular exclusion limit, expectedly it is easier to monitor the volume changes associated with the unfolding, provided the conformation of the unfolded state is stable under the chromatographic conditions of analysis. Here we report the study of the stability of proteins and antibodies in solvents containing guanidine hydrochloride or urea. A 3 µm particle size, 30 nm pore size SEC column with 2500 kDa molecular weight exclusion limit is used for the separation and intrinsic tryptophan fluorescence of the proteins and antibodies is used for the detection by fluorescence detector.

Keywords: Chromatography, HPLC, Protein, Proteomics
Application Code: Genomics, Proteomics and Other ’Omics
Methodology Code: Liquid Chromatography
The mission of the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health is to discover how the environment affects people in order to promote healthier lives. To realize this mission the NIEHS supports a wide variety of fundamental and applied research, including the development of technologies and approaches that can more precisely measure environmental exposures at the level of the individual and characterize how different individuals respond to those exposures with respect to disease risk. Through the Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) programs, NIEHS supports small businesses to develop new tools for exposure assessment, including wearable sensor devices to quantify exposures to multiple environmental exposures in real time with location information. Other exposure assessment approaches include remote sensing technologies and devices that combine analyte detection with other lifestyle factors including physical activity and physiological responses to changing exposure conditions. NIEHS also supports the development of assays and biomarkers to characterize individual responses to environmental stressors, novel methods for measuring internal concentrations of environmental agents, remediation technologies for hazardous sites, and computational approaches to use data from toxicology studies to link chemical exposures to pathway alterations and disease outcomes. Priorities for SBIR/STTR research and technology development for NIEHS will be presented and the application and review process for NIH SBIR/STTR grants will be discussed.

Keywords: Array Detectors, Biological Samples, Biosensors, Chemical
Application Code: Biomedical
Methodology Code: Sensors
Many bioconjugate nanosystems have been developed in analytical chemistry, medicine and pharmacy. Gold nanoparticles (AuNP) are most commonly used in nanotechnology and a number of bioconjugation techniques to advance properties of these nanoparticles has been proposed. The choice of proper bioconjugation approach is a very important issue because it directly affects the orientation, accessibility, and bioactivity of biomolecules immobilized on nanostructures [1]. The aim of this study was to synthesize magnetic gold and polymeric nanoparticles and modify them with antibody molecules or enzyme glucose oxidase in order to develop multifunctional nanoparticles for biomedical application. Magnetic-AuNP (M/AuNP) are suitable for protein immobilization and practical applications in biomedicine. The same surface modification methods as that used for AuNP functionalization can be used; it is very simple to separate M/AuNP from the solution; functionalized nanoparticles are free of reagents used for modification. Advanced properties of M/AuNP will be discussed. Poly(pyrrole-2-carboxylic acid) (PCPy) particles were synthesized in aqueous media by chemical oxidative polymerization of pyrrole-2-carboxylic acid monomers using H2O2. PCPy particles were of 50 – 100 nm in diameter. These particles were modified with glucose oxidase (GOx) via primary amine groups after activation of the PCPy carboxyl groups. The stability of GOx was tested electrochemically. After 18 days the electrochemical signal of electrodes modified with nanoparticles retained 46.3% of the initial activity.

Reference

Acknowledgement: Supported by Research Council of Lithuania.

Keywords: Bioanalytical, Biomedical, Biosensors, Biotechnology
Application Code: Bioanalytical
Methodology Code: Surface Analysis/Imaging
Tissue engineering through directed growth of cells on a biological scaffold has tremendous potential to transform human medicine. A key challenge lies in removing cells and immunogenic material from a donor organ while maintaining scaffold properties that will promote growth of newly seeded autologous cells. Another challenge is to understand how reseeded cell types interact with each other and with the scaffold. We examine both the decellularization and recellularization processes using proteomics on samples from vocal folds, livers, and lungs.

We analyzed decellularized scaffolds and identified, with in-depth coverage, remnant cellular proteins that may be unfavorable, and extracellular matrix (ECM) proteins that may be crucial determinants of directing constructive tissue remodeling. Quantitative analysis of change in individual protein abundance through the decellularization process was performed. The observation that the decellularized scaffolds still contain many cellular proteins, although at decreased abundance, indicates that elimination of DNA does not assure adequate removal of all cellular material, underscoring the need for incorporating proteomics into the decellularization evaluation toolbox.

As for recellularization, using vocal fold mucosa (VFM) as model system, we compared different reseeding approaches, developed a novel strategy employing SILAC to differentiate between proteins that were already in the scaffolds and newly synthesized ones, thereby assessing the active protein synthesis and in vitro remodeling of the ECM. The VFM is an attractive model for matrix remodeling studies because it contains an exquisite ECM that is finely tuned for biomechanical transfer of aerodynamic energy to acoustic energy during voice production. Our study analyzes, for the first time, the dynamic relationship between matrix and growing cells, providing biological system-wide insight into the tissue regeneration process.

Keywords: Biomedical, Characterization, Proteomics, Tandem Mass Spec
Application Code: Biomedical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Investigating the Effects of Commercial Preservative Agents on Human Corneal Epithelial Cell Membranes

Multipurpose contact lens solution (MPS) preservatives have been suggested to cause corneal injury. To determine the validity of this assumption, the molecular-level interactions of common disinfectants in soft contact lens MPS and the corneal epithelium using an in vitro model were assessed. A liposome-based model of the corneal epithelial surface was developed and used to assess the interactions of polyhexamethylene biguanide (PHMB), and polyquaternium-1 (PQ-1) on membrane components and the effects of PHMB and PQ-1 on membrane integrity. Membrane integrity was assessed by measuring the liposome transition temperature using the lipophilic fluorescent probe 6-dodecanoyl-2-dimethylaminonaphthalene (Laurdan). Laurdan is a fluorescent probe that detects changes in membrane phase properties through its sensitivity to the polarity of its environment in the bilayer. Variations in the membrane water content cause shifts in the Laurdan emission spectrum, which are quantified by calculating the generalized polarization (GP). Laurdan fluorescence can then be used to distinguish differences in the membrane order and fluidity by measuring the temperature dependent Laurdan GP. It was found that PHMB improved membrane integrity through interactions with the membrane surface observed as an increase in Laurdan GP and an increase the membrane transition temperature. In contrast, PQ-1 had no effect on membrane integrity as observed by no change in Laurdan GP or the membrane transition temperature. However, upon PQ-1 addition interactions between the membrane surface and PQ-1 caused liposomes to aggregate significantly and precipitate from solution leading to the conclusion that PQ-1-to-liposome bilayer interaction is destructive on a larger scale.

Keywords: Fluorescence, Lipids, Preservatives, Spectroscopy
Application Code: General Interest
Methodology Code: Fluorescence/Luminescence
According to the World Health Organization (WHO), breast cancer is the most fatal form of cancer for women worldwide and attributes to 16% of all female cancers. Although there have been many advances in early detection and treatment, a cure is yet to be discovered. Immunotherapy, which is designed to induce or enhance immune response, is being explored as an alternative to current therapies, including surgical resection, chemotherapy, and radiation therapy. While vaccines are most commonly used prophylactically for several infectious diseases, this study attempts to explore the benefits of a therapeutic cancer vaccine. Here, we will aim to formulate and evaluate a therapeutic breast cancer vaccine using a microparticulate delivery system. The whole cell lysate (WCL) of 4T07 murine breast cancer cells will be incorporated in an aqueous polymer matrix and spray dried with a Buchi B-290 mini spray dryer to formulate an enteric protected vaccine microparticle. The total protein concentration of the WCL will be determined by Bio-Rad DC total protein assay. Additional cell based assays, including MTS cell cytotoxicity assay, phagocytosis studies, and various immune response studies, will be used to evaluate the efficacy of the particulate vaccine. This approach can potentially be translated to a future clinical setting, wherein the patient undergoes surgery and their tumor cells can be used for an individualized particulate vaccine, which can be administered therapeutically to avoid relapse.
### Session Title
Biomedical Applications

### Abstract Title
Synergistic Effects of Plant Extracts and Antibiotics on MRSA Isolated from Clinical Specimens

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### Abstract Text
The study has been carried out in order to evaluate the interaction between different plant extracts individually and in synergy with various antimicrobial agents. Well diffusion method was employed to carry out the anti-MRSA activity of the samples. The zone of inhibition results in mm, showed additive results suggesting the possibility of concurrent use of antimicrobial drugs and plant extracts in combination in treating infections caused by MRSA.

### Keywords:
Natural Products

### Application Code:
Drug Discovery

### Methodology Code:
Other
Photothermal therapy (PTT), as a minimally-invasive therapy, has generated a great deal of interest to kill cancer. Better targeting of photoabsorbers to tumors, has become a new concept in the battles with cancer for improved treatment efficacy and reduced side effects. In this study, the bombesin analog (BBN), with binding ability to all GRP receptor subtypes, was bound covalently with gold nanorods (GNRs) via the nanothink acid as a linker and coated with PEG to increase stability and biocompatibility. The interactions were confirmed by UV-vis and FT-IR spectroscopy. Cytotoxicity study showed biocompatibility of GNR-BBN-PEG conjugate. The cell binding and internalization studies showed high specificity and internalization of GNR-BBN-PEG towards breast cancer cells. The in vitro study revealed destroying of breast cancer cells exposed to new agent and 830 nm continuous wave laser irradiation for 3 min at 80 mW. The biodistribution study showed significant accumulation of GNR-BBN-PEG in breast tumor in mouse model. The in vivo photothermal therapy showed the complete disappearance of xenografted breast tumor in mouse model. The results showed considerable potential of new nanodrug to selective killing of cancer cells in mouse model.
Molecular recognition-based separation techniques have received much attention in chemistry and biology because of their high selectivity for target molecules. Cholesterol, chosen as a target molecule of molecular imprinted membranes investigated in this study, is a major ingredient of the plaque that collects in the coronary arteries and causes coronary heart diseases. Cholesterol imprinted poly(2-hydroxyethylmethacrylate-co-methacryloylaminotryptophan) [p(HEMA-co-MATrp)] membranes were prepared by UV irradiation. Functional monomer MATrp was synthesized by the reaction of L-tryptophan and methacryloyl chloride. 2,2-azobisisobutyronitrile (AIBN) and toluene were used as initiator and pore maker, respectively. Cholesterol imprinted membranes were characterized by elemental analysis, FTIR and SEM. Adsorption experiments were performed in batch experimental set-up in methanol or intestinal mimicking solution. Stigmasterol and estradiol were used as competing molecules in selectivity tests. Obtained some results were as follows: According to the elemental analysis results, with the increase in the amount of functional monomer MATrp in the polymerization medium, incorporation of MATrp was increased. Specific surface area of NIP and MIP particles were found as 13.7 m2/g and 36.5 m2/g, respectively. Template molecules were removed from the membrane in the ratio of 82-88%. Imprinted membranes were 1.96 and 2.13 times selective with respect to the stigmasterol and estradiol, respectively. Imprinted membrane showed negligible loss in the cholesterol adsorption capacity after ten adsorption-desorption cycles with the same adsorbent.

Raman spectroscopy can provide a molecular-level signature of the biochemical composition and structure of cells with excellent spatial resolution and could be useful to monitor changes in composition for early stage and non-invasive cancer diagnosis, both ex-vivo and in vivo. The high wavenumber region (2,800–3,600 cm⁻¹) provides more specific information based on N-H, O-H and C-H vibrations and can be used to identify changes, for example, in lipidic content. It is known that ethanol has a strong effect on the cell membrane, which could directly affect changes in the membrane and cytoplasm lipidic structure and content. In this study, we demonstrate the potential of using the high-wavenumber spectral region of the Raman spectrum to discriminate between oral cells lines treated and non-treated with 1% ethanol. The in vitro Raman spectra were analyzed by the area under the curve as a discriminate method. In this region we discriminated comparing the vibrational modes of CH₃/lipids and OH-confined water bands. Also we were able to demonstrate the effects of ethanol in the cell membrane by using dynamic molecular analysis. These results show the importance of the effects of ethanol for cancer-induced in oral mucosa, which is not completely clear in literature.

Keywords: Biological Samples, Lipids, Raman, Vibrational Spectroscopy
Application Code: Biomedical
Methodology Code: Vibrational Spectroscopy
Use of Raman Spectroscopy for the Study of Cell Cycle Phase and Biomarker Expression Levels in Oral Cancerous Cells

Oral and Pharyngeal cancers are the sixth most common worldwide. 90% of intraoral cancers are squamous cell carcinomas (SCC). They are associated with high morbidity and mortality rates with a 5 year survival of 50%. Oral SCC is often preceded by dysplastic changes in the epithelium and a host of molecular changes such as the inactivation of p53 and overexpression of EGFR and cyclin D1. Cyclin D1 is overexpressed in a number of carcinomas such as breast, oesophageal, ovarian, hepatocellular, colorectal, and head and neck carcinoma. Cyclin D1 overexpression has been associated with increased metastasis and poor prognosis. Raman spectroscopy can be used to determine the biochemical composition of a sample, where each peak corresponds to certain vibrations in molecular bonds. The aim of this study was to investigate Raman spectroscopic profiles and cyclin D1 expression levels in oral cancerous cells. An oral squamous cell carcinoma cell line, SCC4 was used for the study. The cells were synchronized using thymidine and the percentage of cells in each cell cycle phase was determined using flow cytometry. A fluorescently tagged secondary antibody was used to detect the expression of cyclin D1 in the cells. In parallel Raman spectroscopy was carried out on the synchronized cell populations. The results showed that cyclin D1 expression varied at different stages of the cell cycle, with higher expression in G1 and G2/M phases of the cell cycle compared to S phase. Raman spectroscopy could separate the different phases of the cell cycle which we were able to show using unsupervised PCA and linear discriminant analysis utilizing leave one out cross validation.

This work was supported by Science Foundation Ireland.

Keywords: Biomedical, Infrared and Raman, Medical, Vibrational Spectroscopy
Application Code: Biomedical
Methodology Code: Vibrational Spectroscopy
Melatonin played an important role in Alzheimer’s disease as an antioxidant and as a neuroprotector. This study focused on development of the novel sensitive substrate of Fe3O4@Ag core-shell magnetic nanoparticles for melatonin detection. Fe3O4@Ag nanoparticles were synthesized, and their surface morphology and structure were characterized using TEM. The sensitivity and reproducibility of the substrates were tested by detecting rhodamine 6G (R6G), a common SERS probe molecule. More importantly, this substrate showed big advantages of extraction and enrichment of low concentration R6G samples which could further lower the detection limit. This study provides a potential method for analysis of trace amount of melatonin in complex biological samples by combining separation and detection.

Keywords: Bioanalytical, Nanotechnology, Raman, Spectroscopy
Application Code: Biomedical
Methodology Code: Other
Low-Cost Glucometer with Ink-Jet Printed Test Strips

Resource-poor settings have trouble treating diabetes with glucometers because test strips are either unavailable or unaffordable. Some regions receive donated equipment, but each strip type is only compatible with one type of meter. If the donations are not the same every time or are not consistent, they do not meet the need of the patient. A meter without its specific type of strip cannot be used. We have designed a system to help resolve these issues using modified ink-jet printing to create affordable test strips that can be read by a colorimetric glucometer. We fill color cartridges with enzyme and dye solutions and print them onto filter paper backed by contact paper. The filter paper allows for separation of the red blood cells from the plasma in the blood sample, and the contact paper provides wet strengthening for the strips. The reaction between the enzymes and dye (Glucose Oxidase, Horseradish Peroxidase, and 2,2’-Azino-Bis(3-Ethylbenzthiazoline-6-Sulfonic Acid) creates a linear relationship between the absorbance measurement of the test strip and the glucose concentration. We have designed a meter from easily sourced parts that reads the absorbance and calculates the glucose concentration. The strips would be able to be manufactured in resource-poor settings, alleviating the dependence on donations. They are much more affordable as well, costing $0.013 apiece to produce, opposed to the average $1 cost of strips currently on the market. Future work will involve testing using human blood and eventually distribution in Tanzania via our partnership with Madaktari Africa.

Keywords: Bioanalytical, Biomedical, Biosensors, Biotechnology
Application Code: Biomedical
Methodology Code: Sensors
Antimicrobial peptides kill bacteria by disrupting the bacterial cell membrane and it is therefore difficult for bacteria to develop drug resistance. They have been widely researched to be used as new antibiotics and to be immobilized on surfaces to produce effective antimicrobial coatings. In this study, we immobilized an antimicrobial peptide, MSI-78, onto a self-assembled monolayer (SAM) surface via click chemistry. Two mutants, MSI-78 with an azido functionality on either N- (nMSI-78) or C-terminus (MSI-78n), were clicked to alkyne terminated SAMs. Sum frequency generation (SFG) vibrational spectroscopy was applied to characterize the structural information of the surface immobilized MSI-78 via different termini. SFG results showed that both immobilized peptides adopt an alpha-helical structure, but with different orientations, with nMSI-78 standing up and MSI-78n lying down on the surface. Coarse grain molecular dynamics simulations were used to validate the SFG data and provided more detailed understanding on the peptide-surface interaction. The antimicrobial activity tests indicated that the “standing-up” peptides interact with bacterial cells much quicker than the “lying-down”. Such antimicrobial surfaces prepared using immobilized MSI-78 with click chemistry were found to be easy to prepare, store and very stable. We believe that this study provides fundamental insights into how to rationally engineer peptides and substrate surfaces to produce optimized abiotic/biotic interfaces for antimicrobial applications and beyond.

Keywords: Characterization, Immobilization, Peptides, Spectroscopy
Application Code: Biomedical
Methodology Code: Other (Specify)
The objective of this study is to investigate the efficacy of either individual or combined doses of idebenone, carnosine and vitamin E in ameliorating some biochemical indices in liver of nano-sized Titanium dioxide (n-TiO2) intoxicated mice. Nano-anatase TiO2 (21nm) was administered as a daily oral dose of 150 mg/Kg for 2 weeks followed by the aforementioned antioxidants daily either individually or in combination for 1 month. N-TiO2 induced a significant elevation in serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and hepatic oxidative stress biomarker malondialdehyde (MDA) associated with a significant reduction of glutathione (GSH) level, however the hepatic inflammatory markers [Tumor necrosis factor-α (TNF-α) and Interleukin -6 (IL-6)] were augmented by n-TiO2. Caspase-3 level along with Bax gene expression were also enhanced while Bcl2 was down regulated. COMET assay showed DNA damage post n-TiO2 administration. Moreover, mRNA gene expression observed a significant elevation in nuclear factor kappa beta (NF-κB) and nuclear factor relation-2 (Nrf2) .Histopathological examination of hepatic tissue reinforced the previous biochemical results. Co-treatment with either idebenone, carnosine, vitamin E alone or in combination ameliorated the deviated parameters with a variable degrees against n-TiO2 toxicity in mice. In conclusion, our findings suggest that the different treatments exert a therapeutic protective effect in n-TiO2 toxicity by decreasing oxidative stress, mRNA gene expression and hepatic tissues DNA damage.
Cancer treatment currently focuses on radio-, chemo- and immune-therapeutic techniques; however, these treatments also show toxicity toward normal cells. Considering the massive side effects on patients from these treatments, it is essential to develop new techniques that are selective toward cancer cells. Recently, a new group of compounds, i.e. a Group of Materials Based on Organic Salts (GUMBOS), have been designed and are being studied for various applications. These solid phase ionic liquid mimics are also organic salts; however, their melting points make their applications much wider. Synthesis of a range of hydrophobic to hydrophilic GUMBOS was accomplished through anion variations and relative hydrophobicity was measured using octanol-water partition coefficients. Bio-spectroscopic technology was then used for determination of cytotoxicity using cell viability bioassays. Interestingly a trend between cytotoxicity and hydrophobicity was observed with the Rhodamine-6G GUMBOS. Experimental evidence shows that toxicity of the Rhodamine-6G dye towards cancer cell lines as well as normal cell lines are quite different. In this regard, hydrophobic GUMBOS formed nanoparticles which are selectively toxic towards cancer cells with lowered toxicity towards normal cells. Previous research shows that Rhodamine-123, a derivative of Rhodamine-6G, and Crystal Violet are also toxic to cancer cells. This research has advanced to investigating the trend of cytotoxicity and hydrophobicity of Rhodamine 123 and Crystal Violet using the previously determined GUMBOS strategy.

We acknowledge support of an LSU Economic Development Fellowship for support of this research.

Keywords: Biomedical, Biopharmaceutical, Luminescence, UV-VIS Absorbance/Luminescence
Application Code: Biomedical
Methodology Code: Biospectroscopy
Pancreatic cancer is one of the deadliest cancers with a mean survival time of few months: 4-8 months for more than 80% of patients. The main reasons of this poor prognostic are the absence of specific symptoms and the lack of biomarkers, leading to a late detection. Aptamers are single stranded DNA oligonucleotides which bind to their target with good specificity and high affinity. They are generated through a process name cell-SELEX (cell based Systematic Evolution of Ligands by EXponential Enrichment). The principle of SELEX is the evolution of an initial random library of $10^{15}$ DNA sequences through successive rounds until the binding sequences ($10$ sequences) are enriched and a panel of aptamers is generated. Once generated, those probes need to be characterized in term of binding affinity, specificity as well as the potential to get internalized. The main advantage of cell-SELEX is the generation of a probe to recognize a whole cell instead of a specific protein removing the need of knowing the target before starting the selection and leading to the discovery of new biomarkers. In this study, these probes have been developed to recognize pancreatic ductal adenocarcinoma and they have been characterized. In brief, the generation of new molecular probes to recognize pancreatic cancer cells is required in order to detect and treat more efficiently this disease.

This work is supported by the National Institutes of Health (GM079359 and CA133086).
Carnitine palmitoyltransferase 1 (CPT1) catalyzes the rate-limiting step in long chain acyl-CoA's transport from cytoplasm to the mitochondrial matrix. There are 3 CPT1 isoforms: CPT1A is mainly in liver and it is located in the outer mitochondrial membrane (OMM). CPT1B is located in the OMM of muscle and brown adipose tissue and CPT1C is expressed in brain and testes, and its location is ER. Their structures are not described as they have not been crystallized yet. Studies about their quaternary structure using chemical cross-linking methodology suggest that CPT1A self-assembles into an oligomeric complex of trimers, which associates into a dimer to yield hexamers. Nothing is claimed about CPT1C's disposition in the ER.

Our objectives are:
1. Confirm CPT1A's conformation in the OMM.
2. Define if CPT1C adopts the same polymeric organization as CPT1A.
3. Study CPT1A-CPT1C's interactions in contact sites between mitochondria and ER.

To achieve these objectives, we designed recombinant CPT1 proteins bound to fluorophores in the C-terminal region. Different tones of fluorophores were used: turquoise (Turq2) and yellow (SYFP2). We obtained four plasmids: hCPT1A-Turq2, hCPT1C-Turq2, hCPT1C-SYFP2, hCPT1A-Turq2-hCPT1C-SYFP2. We confirmed their correct sequence by sequencing all the constructs. We also found the best transfection conditions for the chimeric protein's expression in HEK293A cells. Analysis of the transfections by confocal microscopy confirmed their correct protein expression. Transfected cells are ready to be analyzed by AFRET technology to obtain the distances between fluorophores and determine CTP1A and CPT1C's structures and interactions. AFRET's assay is being carried out by Pilar Lillo's group in Rocasolano Biological Physicochemical Institute (Madrid).

Our future work is immunoassay with endogenous CPT1A and CPT1C and study in which pathological conditions CPT1C migrates to OMM.

Keywords: Biological Samples, Biospectroscopy, Characterization, Fluorescence
Application Code: Biomedical
Methodology Code: Other
Biomedical Applications

Structural Identification and Bioactivity Testing of Gold Nanorods Conjugated with Doxorubicin and cRGD for Combined Anti-Cancer Drug Delivery

Chemotherapeutic Drugs with Low aqueous solubility, High systemic toxicity, Lower cellular entry, and Instability at physiological pHs. However the advantages of cancer nanotechnology Nanotechnology may also be useful for developing ways to eradicate cancer cells without harming healthy, neighboring cells. Scientists hope to use nanotechnology to create therapeutic agents that target specific cells and deliver their toxin in a controlled, time-released manner. Highly selective targeting to cancer cells without harming healthy cells; Nanosystem can do much more than deliver treatment. NIR spectral region is ideal for in vivo imaging. Multifunctional nanosystem offer more powerful outcomes for cancer therapy. They can do much more in an all-in-one system: 1) assist in imaging inside the body; 2) recognize precancerous or cancerous cells; 3) release a drug that targets only those cells, and 4) report back on the effectiveness of the treatment. Gold Nanorods Conjugated with Doxorubicin and cRGD [cyclo(Arg-Gly-Asp-D-Phe-Cys)] for Combined Anti-cancer Drug Delivery and PET Imaging Synthetic scheme for the multifunctional GNR-DOX-cRGD-64Cu nanocarriers.

It is expected that most DOX conjugated onto the system would not be released prematurely into the bloodstream (pH 7.4) before reaching the targeted tumor sites, yet provide a sufficient amount of drug to effectively kill the cancer cells once the nanosystems are internalized and enter the endocytic pathway, thereby greatly enhancing the therapeutic efficacy while reducing the undesirable side effects. The level of cellular up-take of the GNR nanocarriers or free DOX directly correlated with cytotoxicity. Increasing cell uptake using cRGD peptides conjugated onto the GNR nanocarriers to target aVb3 integrin on tumor cells can directly increase cellular destruction.

Keywords: Biomedical
Application Code: Biomedical
Methodology Code: Other
Fuels, Energy & Petrochemical

The Determination of Mercury in Unstabilized Hydrocarbon Liquid Streams by Vaporization-Amalgamation-Atomic Fluorescence Spectrometry

Unstabilised hydrocarbon liquid streams such as unstabilised condensate present particular challenges for sampling and determination. If the liquid sample is depressurised, a portion of the sample is vaporised, producing a two phase sample for determination. Mercury can be associated with both the gas and liquid portions of the sample. Conventionally, determination of liquid process streams which are gas-liquid mixtures when depressurised is difficult due to the need to characterise two separate phases and combine the results.

This poster describes a novel offline auto-injection system designed to introduce the sample to the analyser at process pressure to avoid the issues associated with depressurisation. Sample is collected into a sample vessel (or “bomb”) at process conditions. The vessel is then connected to the auto-injection system where it is pressurised with nitrogen to ensure it remains 100% liquid. A pressurised sample flows through an injection valve, where aliquots of sample are introduced to the vaporisation chamber of a pre-concentration unit, operated in conjunction with an amalgamation-atomic fluorescence spectrometry analyser.

This poster further describes a similar online implementation, using ATEX/IECEEx zone 1, certified equipment. The system can be configured for up to eight sample streams. Filtered sample is delivered at process pressure to an injection valve, where aliquots of sample are introduced to the vaporisation chamber of a pre-concentration unit and amalgamation AFS analyser. Both the Pre-concentration unit and analyser are located in purged enclosures.

Results will be presented for demonstrating both the offline and online approaches.

Keywords: Atomic Spectroscopy, Hydrocarbons, Mercury, Petrochemical

Application Code: Fuels, Energy and Petrochemical

Methodology Code: Atomic Spectroscopy/Elemental Analysis
In order to accurately assess battery safety in a particular application, a safety evaluation needs to use realistic application scenarios including power drain, charge modes, duty cycle, and so on. Adiabatic calorimeters accurately provide “worse case” or “real life” measurements which is why they are commonly used in safety applications to study and prevent runaway reactions. In addition, the ability to safely and accurately measure the heat changes associated with the charging and discharging process is key to battery management system (BMS) design and for correct thermal management. Isothermal Calorimetry, on the other hand, is the best approach to get this information for large lithium ion batteries used in EV applications as other approaches simply do not scale appropriately for these larger systems. Cycling a cell inside a calorimeter can provide information on the efficiency of the battery and the effect of added connections and other support electronics, such as the BMS, to larger modules and packs. This poster will compare both Adiabatic and Isothermal Calorimetry methods as applied to lithium ion battery characterization with regards to their safety and performance aspects.
Formic acid (HCOOH) has been considered a promising fuel in power technologies, for its low toxicity, low crossover, good electrical conductivity, high open circle potential, and availability from biomass. However, effective electro-oxidation of HCOOH requires the use of highly efficient anodic catalysts. We report here the syntheses of four palladium (Pd) based nano-materials and their catalytic activities towards the electrochemical oxidation of HCOOH. The first and second Pd nano-sized catalysts were prepared by chemical reduction using NaBH₄ of PdCl₂ mixed with graphite nano-particles (GNP) (~10 nm) and graphite powders (C) (20-50 nm), respectively. The as-prepared Pd/GNP and Pd/C catalysts were casted on glassy carbon electrodes (GCE) with Nafion separately, and used for HCOOH oxidation. The Pd/GNP catalyst prepared from 1 to 4.2 molar ratio of PdCl₂ over GNP showed a broad electrochemical oxidation peak at ~+0.30 V vs Ag/AgCl (3.0 M KCl) in 0.10 M HCOOH-0.10 M H₂SO₄. However, when the molar ratio of PdCl₂/GNP and PdCl₂/C changed to 1/1.7, the particles showed greater stability with oxidation peaks at ~+0.13 V for Pd/GNP and ~+0.18 V for Pd/C nanocatalysts, respectively. The morphology (SEM images) and energy-dispersive X-ray spectroscopy (EDX analysis) were also reported for both the catalysts. Additionally, 20 wt% Pd nano-materials on three different carbon based support materials (GNP, Vulcan XC-72, and Ketjen Black) were synthesized. The prepared catalysts were compared with the commercial 20 wt% Pd/activated carbon for formic acid electrooxidation using Hg/Hg₂SO₄ (satd. K₂SO₄) with a 0.025 mg Pd/cm² of GCE as catalytic load. The stripping peaks at ~+0.05 V vs Hg/Hg₂SO₄ (satd. K₂SO₄) were also explained in terms of change in scan rates as with slower scan rates (10 mV/s and below) the peak disappearance which corresponds to the reduction of PdO to Pd. Furthermore, the reference electrode was changed from Ag/AgCl (3.0 M KCl) to Hg/Hg₂SO₄ (satd. K₂SO₄) as the typical formic acid oxidation behavior was not observed which is under investigation.

Financial support from the CAREER Award (CHE-0955878, WJM) is gratefully acknowledged.

**Keywords:** Electrochemistry, Energy, Fuels\Energy\Petrochemical, Nanotechnology

**Application Code:** Fuels, Energy and Petrochemical

**Methodology Code:** Electrochemistry
Hydrogen measurement is critical to the control of a number of modern refining and chemical processes. Widespread deployment of hydrogen measurement has been impeded by harsh process conditions that rapidly degrade common low cost hydrogen sensors. In this paper we present a novel sensing technology that overcomes these limitations and explore some of the applications that are thus enabled.

**Abstract Text**

Hydrogen measurement is critical to the control of a number of modern refining and chemical processes. Widespread deployment of hydrogen measurement has been impeded by harsh process conditions that rapidly degrade common low cost hydrogen sensors. In this paper we present a novel sensing technology that overcomes these limitations and explore some of the applications that are thus enabled.

**Keywords:** Electrochemistry, Energy, Fuels\Energy\Petrochemical, Gas

**Application Code:** Fuels, Energy and Petrochemical

**Methodology Code:** Sensors
TD-NMR Combined with Chemometrics Analysis: An Alternative Tool for Monitoring Diesel Fuel Quality

Time domain Nuclear Magnetic Resonance (TD-NMR), based on low cost benchtop instrument, has become an attractive alternative for traditional analytical methods due to the ability to analyze samples with little or no sample preparation, fast data collection and capability to serve as inline measurements. These advantages result in time and cost savings and higher analytical throughput. In this context, the aim of this study was to apply TD-NMR combined with chemometrics analysis to classify and predict quality parameters of Brazilian commercial diesel samples. Diesel samples were provided by the Center for Characterization and Development of Materials (CCDM, São Paulo, Brazil). Cetane index, density, flash point and temperature achieved during distillation to obtain 50% of the sample distilled (T50) reference values were obtained according to the Brazilian standard methods (ABNT NBR). TD-NMR experiments were evaluated in the SLK 100 TD-NMR benchtop instrument (Spinlock Magnetic Resonance Solution, Argentine) equipped with a 0.23-T permanent magnet. 1H transverse relaxation times, $T_2$, were performed using Carr-Purcell Meiboom-Gill (CPMG) pulse sequence with a $90^\circ$ pulse width of 6.4 $\mu$s, time between echoes of 2 ms, 1000 echoes and a recycle delay of 1.5 s. Soft independent modeling of class analogy (SIMCA) and partial least squares regression (PLS) was applied to generate classification and calibration models, respectively. SIMCA models exhibited tight and well-separated clusters, allowing the discrimination of the diesel samples based on the sulfur content: 10 (S10), 500 (S500) and 1800 (S1800) mg kg$^{-1}$. PLS models showed good agreement between the real and predicted values, with high coefficients of correlation ($r > 0.98$) and low standard error of prediction (SEP). These results support the application of TD-NMR to evaluate the quality of diesel, providing simple, rapid and non-destructive method for the petrofuel industry.

Keywords: Chemometrics, Fuels\Energy\Petrochemical, NMR
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Magnetic Resonance
In this work, several experiments for characterization of the main wastes generated by sugar and ethanol productions from sugar cane (S. officinarum) were conducted. Such wastes (bagasse, filter cake and vinasse) were sampled from two sugar cane mills located in a radius of 170km from Araraquara, Brazil. Analytical and thermoanalytical techniques such as ultimate analysis, determination of higher heating value (HHV), X-ray diffractometry (XRD), infrared spectroscopy (FTIR), atomic absorption and emission spectrophotometry (AAS and AES, respectively), vis-spec, thermogravimetric analysis and differential thermogravimetric analysis (TGA-DTG), differential thermal analysis (DTA) and differential scanning calorimetry (DSC) were employed. In TGA-DTG and DTA, dynamic atmospheres of synthetic air and N\textsubscript{2} were used. DSC curves were conducted in a static air atmosphere. Despite similar organic constitution, differences in HHVs and in thermal behavior were observed due to high contents of inorganic matter detected in filter cake and vinasse. Thus, a higher amount of ash was generated when such wastes were heated in relation to bagasse. Among samples of the same waste from different sugar cane mills, changes in their constitutions were detected, mainly regarding to metals concentration (Ca, Cr, Fe, Mn, Ni and Pb). Therefore, for a same waste, different thermal behaviors were observed for each sample in terms of displacement of peaks to lower temperatures and maximum thermal decomposition rates.

The authors would like to acknowledge CAPES for financial support.
Thermogravimetric analysis (TGA) is an analytical technique in which changes in physical and chemical properties of materials are measured as a function of temperature and/or time. TGA is commonly used to determine selected characteristics of materials that exhibit either mass loss, or gain, due to decomposition, oxidation, or loss of volatile material, such as moisture. Common applications of TGA are materials characterization through analysis of characteristic decomposition patterns and determination of combustible materials and combustion residues. Macro TGA systems that use gram-size samples have been used for a couple of decades for monitoring industrial processes. The larger sample sizes allow more accurate mass measurements and a representative sample for characterization of heterogeneous materials. Flue gas desulfurization (FGD) solids are industrial byproducts that have been monitored using macro TGA systems. Compounds reported to have been successfully measured include free moisture, moisture in hydrates, gypsum, lime, CaSO$_3$·0.5H$_2$O, and CaCO$_3$. The actual measurement of CaSO$_3$·0.5H$_2$O in FGD solids is questionable and is the subject of this study.

A LECO TGA701 macro TGA system and elemental analysis data were used in a study to characterize the components in various FGD solids. Pure materials, and their mixtures, were first analyzed with the TGA701. Pure CaSO$_3$·0.5H$_2$O is not available and was not used in the study. The study of pure materials and mixtures demonstrated the accuracy of the TGA701 in characterizing the compounds. In most of the previously reported macro TGA studies of FGD solids, lime is not reported as one of the components in FGD solids. The TGA reaction profiles for lime and CaSO$_3$·0.5H$_2$O decomposition occur in the same temperature range. This study sheds new light on this subject.

Keywords: Energy, Environmental/Waste/Sludge, Thermal Analysis
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Thermal Analysis
Viscosity Reduction of Heavy Crude Oils Using Hydrogen or Methane as Reducing Agents

Heavy crude oils are an important part of the world reserves of liquid hydrocarbons. In offshore fields, where the production of these crude oils is limited by their high viscosities, viscosity reduction via crude oil upgrading is not feasible due to the size and cost of the facilities for hydrogen production or for high-carbon-content residues handling. In this work, we evaluate the alternative of using methane as reducing agent instead of hydrogen in the mild thermal cracking of a heavy crude oil.

Experiments were carried out in a pilot plant at 713 K and 56 bar. The flows of heavy crude oil (12.7 °API, 1101 cSt @ 333 K, 48.4 vol. % total distillates to 811 K) and hydrogen or methane were controlled using mass flow controllers. The distillation curves of the heavy and upgraded crude oils were determined by the ASTM D7169 GC-Simulated Distillation method, the viscosities by the ASTM D-7042-04 method (Stabinger viscometer) and the sediments contents by the ASTM D-4807-05 method.

For the test using hydrogen as reducing agent, total distillates yield to 811 K in the upgraded crude oil was 64.4 vol. %, viscosity was 57 cSt and sediments content was 0.4 wt %. For the test using methane, total distillates yield was 62.0 vol. %, viscosity was 115 cSt and sediments content was 0.8 wt %.

These results show that hydrogen is appreciably more effective than methane for preventing the polymerization of heavy molecules in the free radicals reactions in thermal cracking.

Keywords: Characterization, Fuels\Energy\Petrochemical, Gas Chromatography, Petroleum

Application Code: Fuels, Energy and Petrochemical

Methodology Code: Gas Chromatography
Meeting Novel Challenges in Specialty Gas and Petrochemical Applications with GC Plot U and Select Low Sulfur Plot Columns

Recent column manufacturing improvement and new application specific designed stationary phases for GC PLOT columns make it possible to look at separations in new ways. Separations that have historically required more than one column and/or multiple methods can be simplified by taking advantage of recent improvements in Plot column technology. Approaching challenging separation form a new prospective is leading to successful single column solutions on these phases.

The separation of hydride impurities in Arsine, Phosphine, Propane and Propylene are promising using Pora Plot U columns showing excellent separation and low level detection by GC-ICP-MS. Using new detection and separation tools to revisit long standing challenges such as trace level analysis of arsine in phosphine matrices is bearing fruit. Example chromatograms highlighting peak shapes and low level detection illustrate the power or revisiting long standing challenges with better analytical tools.

Keywords: Capillary GC, Fuels\Energy\Petrochemical, ICP-MS, Sulfur
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography
On-Site Rapid Analyses of Well Gases for Mud Logging Applications Using Micro Gas Chromatography

Oil and gas exploration require the analysis of dissolved natural gas in mud samples from the well within short run times. This posters highlights the use of an Micro Gas Chromatograph for rapid, accurate mud logging analysis. Gas chromatography is proven to be an accurate and sensitive technique for the characterization of individual hydrocarbon gases to combine in lithology reports for the mud logging field. Critical information is obtained for making decisions on additional drilling or production of the well.

A system equipped with two analytical channels is used for on-line analytical testing of drilling fluid samples. Each column channel is a complete GC containing an electronic carrier gas control, micro-machined injector, narrow-bore analytical column and micro thermal conductivity detector (TCD). Dissolved gases, collected from the drilling fluid samples using a semipermeable membrane, are analyzed on both analytical channels in just over 30 seconds.

Miniaturization has resulted in a small, shoe-box size, instrument dimensions that makes it easy to integrate into on-site control cabins or explosion proof enclosures. Moreover, industry standard 19-inch rack configuration further simplifies integration into mud logging operations.

Keywords: Fuels\Energy\Petrochemical, Gas Chromatography, Portable Instruments, Process Monitoring
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography
### Abstract Text

Adsorbent materials are widely used in the oil and gas sector to remove mercury from gas and liquid feeds. Similarly, adsorbent materials are also used in the treatment of waters and waste waters to remove mercury, arsenic and selenium, amongst other contaminants. The performance of these adsorbent materials varies significantly with the physical and chemical nature of the material itself and with the composition and flow rates of the fluid stream being cleaned.

There is a significant requirement within the industry to test and demonstrate the performance of adsorbent media, from both the suppliers of the media and the end users. Generation of the required breakthrough curves is time consuming and potentially labour intensive. The use of a suitable automated test system facilitates the collection of suitable data to demonstrate/test the performance of materials.

Working with standard modules, with bespoke components where required, test bed systems have been developed for a range of applications. Systems can work with either gaseous, aqueous or hydrocarbon liquid. In all cases the bed flow rates are precision controlled with mass flow controllers or metering pumps and the media volume recorded. Analyte concentrations are automatically measured at the inlet, mid-bed and outlet points, with multiple parallel bed streams accommodated to reduce total testing time. A range of additional criteria can also be met, including wide concentration ranges and addition of other contaminants.

This poster describes bespoke systems for testing adsorbent materials in gaseous, aqueous and hydrocarbon liquid media, presenting breakthrough curve data for various adsorbents.

### Keywords

- Automation, Environmental/Water, Hydrocarbons, Mercury
- Fuels, Energy and Petrochemical
- Atomic Spectroscopy/Elemental Analysis
Advances in a New Methodology for Sampling and Analyzing Elemental Sulfur in Natural Gas and Natural Gas Liquids

The presence of even small amounts of elemental sulfur in natural gas is known to facilitate corrosion in distribution lines, increasing the costs of maintenance and concerns over safety. If the sulfur travels through the system to the final user, the environmental and health problems can be severe. The sulfur fumes can cause irritation of the eyes, nose, and respiratory tract. Elemental sulfur can react with O2 to form SO2, which can cause vascular damage in the brain, heart and kidneys, as well as imbalance the enzyme system. It is clear the importance of finding a way to detect and quantify elemental sulfur in the natural gas. Over the last couple years, we have been working on a new approach to fulfill this need: a regular sample cylinder is used as a base for a simple mechanical trap to collect the sulfur from a known amount of gas. This cylinder is washed with a known volume of a solution of triphenylphosphine and n, n-dimethylformamide in a blend of aromatic solvents, to get the sulfur in a stable organic compound: (C6H5)3PS. This solution has been analyzed with a series of analytical methods, the results of which have been presented previously. In working with our customers on this technique, we have considered the possibility that the sulfur tends to concentrate in the liquid phase associated with the natural gas, and is pushed through the process with it.

In this work, we present the results of this investigation and the latest advances to a definitive method.

Keywords: Gas, Gas Chromatography, Sampling, Sulfur
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Sampling and Sample Preparation
Abstract Text
Diesel fuels are known to generate reactive and potentially harmful compounds upon combustion. In an attempt to compare the profiles of pollutants emitted from Yanmar power generator fueled with diesel and biodiesel blended with different biomass-derived additives, Tedlar™ bag samples of emissions from different fuels were analyzed by GC-MS with a pre-concentrator outfitted with a glass bead trap and a Tenax™ TA trap. The emission samples were also introduced into a 10-meter gas cell for quantitative determination of carbon monoxide, formaldehyde, methane, and ethylene by FTIR spectrometry. The FTIR and GC-MS techniques are complementary to one another because the former technique allows the measurement of C1-C2 compounds at the 0.1-100 ppmv levels and the latter is more suitable for analysis of C3-C12 compounds at the 0.1-100 ppbv levels.

Biodiesel fuels produced with both sodium hydroxide and barium hydroxide catalysts were also compared in terms of their emission profiles as well as their biodiesel yields. Experimental variables of alcohol-to-oil ratio, reaction time, reaction temperature, and catalyst concentration will be presented. The FTIR tests showed significant reduction in the emission of carbon monoxide and formaldehyde for oxygenated biodiesel based on fatty acid methyl esters and glycerol-derived oxygenated additives. Likewise, the production of long-chain aliphatic aldehydes and alcohols along with ketones with less than six carbon atoms were significantly reduced in biodiesel and glycerol-derived additives.

Keywords: Biofuels, GC-MS
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography/Mass Spectrometry
Fuels, Energy & Petrochemical

**Abstract Title**
Trace Detection of Carbon Monoxide in Hydrocarbon Feedstock Processing Using Continuous-Wave Cavity Ring-Down Spectroscopy (CW-CRDS)

**Primary Author**
Florian Adler
Tiger Optics

**Co-Author(s)**
Elyse Baroncini, Erika Coyne

**Abstract Text**
Tiger Optics has developed a new generation of cavity ring-down spectrometer – the CO-rekt[TM][/sup] – for measurement of carbon monoxide, and other gases, in hydrogen. The analyzer is packaged in a NEMA enclosure to allow efficient purging, satisfying Class I, Division 2 regulations, and thus is suitable for operation in hazardous locations. It has been quickly embraced as a gamechanger in the hydrocarbon feedstock world, as it overcomes costly and potentially dangerous issues associated with incumbent technology. Specifically, the device is insensitive to vibration, free of drift, solid state and automatically self-calibrating.

Detection of carbon monoxide in hydrogen is required in a wide range of applications, including process monitoring in HyCO and Steam Methane Reformer (SMR) plants. Such plants mainly serve refineries and chemical industries, and, therefore, need to produce high purity H[sub][2]/sub] to protect their customers’ processes. Continuous, online monitoring of CO is especially critical as it poisons the catalytic beds used to manufacture pure H[sub][2]/sub].

Continuous-Wave Cavity Ring-Down Spectroscopy (CW-CRDS) is a field-proven analytical technique for trace gas detection that directly derives the absolute optical loss due to molecular absorption inside the cavity from a simple time-based measurement, independent of laser intensity noise and environmental conditions. CW-CRDS provides an ideal analytical solution that is fast, sensitive, accurate yet extremely robust and simple to operate.

Performance data will be presented illustrating sensitive detection limits of 100 parts-per-billion of CO in H[sub][2]/sub] and showcasing the analyzer’s wide dynamic range of up to 2000 parts-per-million. Furthermore, the analyzer responds within seconds of a CO excursion event, making it an ideal choice for monitoring process upsets.

**Keywords:** Hydrocarbons, Molecular Spectroscopy, Petrochemical, Trace Analysis

**Application Code:** Fuels, Energy and Petrochemical

**Methodology Code:** Molecular Spectroscopy
India is rich in coal bed methane resources but still lacks in methane development technologies. To begin understanding this prospective source, in the present investigation, microbial methane production was examined from bituminous, subbituminous and lignite coals obtained from Jharia coal mines in India. Microbial populations were enriched over a period of 21 days on various methanogenic specific substrates including the three ranks of coal. Maximum methane production (49%) was obtained on Methanosprillum sp specific medium (sodium acetate and isopropanol) supplemented with subbituminous coal at 65°C and pH 6.8. Phylogenetic analyses of 16S rRNA gene sequences from the selected CBM65 consortium revealed a syntrophic association between a hydrogenotrophic methanogen Methanoculleus thermophiles and fermentative bacteria Comamonas sp. The results of this study shed light on the fact that Jharia coal mines are methanogenically active and offer a prospective source for coal bed methane extraction. Also, considered among the richest source of methane in India this data will help in determining the potential role of methane emitted from this site in influencing the global carbon cycle.

**Abstract Text**

India is rich in coal bed methane resources but still lacks in methane development technologies. To begin understanding this prospective source, in the present investigation, microbial methane production was examined from bituminous, subbituminous and lignite coals obtained from Jharia coal mines in India. Microbial populations were enriched over a period of 21 days on various methanogenic specific substrates including the three ranks of coal. Maximum methane production (49%) was obtained on Methanosprillum sp specific medium (sodium acetate and isopropanol) supplemented with subbituminous coal at 65°C and pH 6.8. Phylogenetic analyses of 16S rRNA gene sequences from the selected CBM65 consortium revealed a syntrophic association between a hydrogenotrophic methanogen Methanoculleus thermophiles and fermentative bacteria Comamonas sp. The results of this study shed light on the fact that Jharia coal mines are methanogenically active and offer a prospective source for coal bed methane extraction. Also, considered among the richest source of methane in India this data will help in determining the potential role of methane emitted from this site in influencing the global carbon cycle.
Oil shale is a fine grained sedimentary rock that contains a solid mixture of organic chemical compounds called kerogen. Oil shale was formed from the organic debris millions of years ago. When heated by natural geothermal heat these shales produce liquid organic products by thermal decomposition.

With the conventional oil resources being quickly depleted, there has been a great boom on the exploration and production of shale oils around the world. One of the traditional methods for identifying shale oil deposits is vitrinite reflectance (VR), which uses light reflectance as an indicator of maturity in hydrocarbon source rocks. However, this technique has several disadvantages, including analysis being subject to human error, low/absent reflectance values in some of the oil shale deposits, etc. Several previous studies indicated that Raman spectroscopy of carbonaceous materials can be used for measuring the thermal maturity. In this study, oil shale samples from Texas were analyzed with a fast imaging confocal Raman microscope and demonstrated that Raman imaging provides a non-biased chemical metric for fast identifying thermal maturity (and oil content) of the oil shales. In addition, Raman imaging also identifies, with high speed and accuracy, the presence of different minerals in the oil shale samples, including calcium carbonate (calcite), quartz, and clay minerals among others. Knowledge of the presence or absence of these minerals in oil shale is essential for efficient oil extraction in drilling operations by the new technique of hydraulic fracturing.
Elemental and isotopic analysis is a key tool for characterising coal material. Using the automated Flash 2000 Elemental Analyzer interfaced with a Delta V Isotope Ratio Mass Spectrometer (IRMS), rapid, accurate and precise Carbon, Nitrogen, Hydrogen, Sulfur, and Oxygen elemental and isotopic data can be obtained. The elemental data combined with the relative heat value calculation allows an assessment on quality of the coal whereas isotope ratio values allow an evaluation of material provenance and diagenetic processes.

The EA system is based follows the Dumas combustion principle and allows simultaneous CHNS determination in a single analysis run and the Oxygen determination by pyrolysis in a second run. The Heat Value and the CO2 emission trade are calculated automatically by the dedicated software. The combustion method is described in ASTM D5373-02, which was followed here.

In this paper we present elemental and isotopic CHNS/O data, Heat Value and a CO2 emission trade calculation on a range of coal samples and demonstrate the reproducibility on the EA and EA – IRMS data.

**Keywords:** Coal, Elemental Analysis, Isotope Ratio MS, Materials Characterization

**Application Code:** Fuels, Energy and Petrochemical

**Methodology Code:** Other
While hydraulic fracturing (fracking) has made shale gas accessible and economical, large quantities of wastewater are produced over the lifespan of a well: drilling and fracturing a Marcellus shale well requires approximately 14,000-17,000 m$^3$ of water. Within one year of well completion, 10-25% of the water returns to the surface as a concentrated brine. Although 55-80% of this returned water is reused for other wells, a substantial amount of wastewater is leftover. These large wastewater volumes have prompted the need for appropriate management and monitoring techniques. Current methods for water analysis are not designed for fracking formulations, thus new approaches are needed to assess and maintain environmental compliance and safety as these valuable natural resources are utilized.

To meet these demands, microfluidic tools are being designed, built and applied. These offer the advantages of low cost, simplicity, stability and high adaptability for widespread distribution. Our devices can be interfaced with multiple detection methods, including colorimetric detection using stand-alone, low-cost hardware and the optical hardware on smartphones. Detection of constituents such as bromide, strontium and barium are of particular interest because they are characteristic of the wastewater generated by Marcellus shale gas extraction. Detection can serve as an early indication of wastewater leaks—a crucial tool for preventing source water contamination. Additionally, rapid detection of these elements can be useful for tracking the movement of produced waters, an important process for ensuring safe wastewater disposal. We will present recent outcomes from our work on quantification of fracturing fluid indicators.

**Keywords:** Environmental/Water, Fuels\Energy\Petrochemical, Lab-on-a-Chip/Microfluidics, Petroleum

**Application Code:** Fuels, Energy and Petrochemical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Fuels, Energy & Petrochemical

Dissolved Gas Determination Using the D-19 ASTM Method

In recent years, there has been a marked increase in hydraulic fracturing or “fracking” in order to recover natural gas from deep beneath the earth’s surface. As a result of this drilling, there has also been a rise in the concern of gases escaping during the “fracking” process and contaminating nearby water sources. In order to test for the possible dissolved gas contamination, most laboratories refer to a standard operating procedure entitled “RSK-175”. This procedure calls for static headspace sampling of the water samples and calculating the amount of dissolved gas using Henry’s Constant. However, as RSK-175 is standard operating procedure and not a formal method, interpretations can vary. In order to address this issue, a formal method is currently being written by American Society for Testing and Materials (ASTM) Committee D-19. This application will test for the dissolved gases using the procedures established in the ASTM method.

Keywords: Environmental/Water, Fuels\Energy\Petrochemical, Gas Chromatography, Headspace

Application Code: Fuels, Energy and Petrochemical

Methodology Code: Sampling and Sample Preparation
Fuels, Energy & Petrochemical

**Abstract Title**

**Porphyrin-Based GUMBOS and NanoGUMBOS for Use as Sensitizers in Dye-Sensitized Solar Cells**

**Primary Author**

Paulina E. Kolic
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**Co-Author(s)**

Bishnu Regmi, Isiah M. Warner, Noureen Siraj, Xinning Luan, Ying Wang

**Abstract Text**

Dye-sensitized solar cells (DSSCs) are low cost, third generation solar cells. In order for DSSCs to be competitive with current technology, the efficiency of DSSCs must be improved. The photosensitizing dye plays a critical role in DSSCs by absorbing photons from sunlight and transferring electrons to generate current. In this regard, a new class of materials known as a Group of Uniform Materials Based on Organic Salts (GUMBOS) and nanoparticles derived from GUMBOS (nanoGUMBOS) is evaluated as photosensitizing dyes in DSSCs. GUMBOS are solid organic salts that are composed of bulky organic ions with a melting point in the range of 25-250°C. The properties of GUMBOS can be tuned by varying the cation and anion, resulting in a unique material possessing characteristics of both ions. NanoGUMBOS retain the beneficial properties of both GUMBOS and nanomaterials. In this study, GUMBOS were synthesized using porphyrin dyes that contain different central metal atoms as anions, and their optical and electrochemical properties were studied using ultraviolet-visible absorbance spectroscopy, fluorescence spectroscopy, and cyclic voltammetry. NanoGUMBOS were prepared by reprecipitation to yield uniform particles between 50 – 100 nm in size. The solar efficiency was investigated using GUMBOS and nanoGUMBOS of differing composition.

We would like to acknowledge NSF CHE–1307611 and DOE-SCGF DE-AC05-06OR23100 for funding.

**Keywords:** Fluorescence, Fuels\Energy\Petrochemical, Microscopy, UV-VIS Absorbance/Luminescence

**Application Code:** Fuels, Energy and Petrochemical

**Methodology Code:** Fluorescence/Luminescence
Porous silicon (pSi) exhibits strong, visible photoluminescence (PL) at room temperature. One negative aspect of as-prepared, H-passivated pSi (ap-pSi) is that the PL is unstable. One approach to stabilize the pSi PL and simultaneously impart chemical functionality to the surface is oxidization to create oxidized porous silicon (ox-pSi) and subsequent silanization with an organically modified silane (e.g., (RO)3Si-R’).

Direct application of candidate silanizing agents to ox-pSi can be rapidly carried out by contact pin-printing (CPP) methods. Towards this end, we have prepared microarrays by CPP using two common silanes: 3-aminopropyltriethoxysilane (APTES) and butyraldehydriethoxysilane (BATES). Initially, fabrication of high density microarrays proved difficult because of significant feature “spreading”. A combined PL and IR imaging study allowed us to determine the root causes of the spreading. We then developed and evaluated new strategies to mitigating feature spread. In the end, although APTES is by far the more popular silane for surface modification and secondary grafting, it may be far less suitable for CPP on ox-pSi if the goal is to form high density microarrays.
### Session Title
Molecular Spectroscopy Advances

### Abstract Title
Characterization of Food and Pharmaceutical Packaging by Molecular Spectroscopy

<table>
<thead>
<tr>
<th>Primary Author</th>
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#### Date: Tuesday, March 10, 2015 - Morning
#### Time: 
#### Room: Exposition Floor, Hall F, Aisles 390

### Abstract Text
Food and pharmaceutical packaging materials are complex multicomponent structures that are designed to fulfill many requirements. They must be durable enough to permit products to be transported great distances. They must block the ingress of moisture, ultraviolet radiation, microbes, and gases to prevent product spoilage and contamination. The packaging must also facilitate sale of the product through compatibility with the printing processes deployed to inform or attract the consumer of the product. FTIR, FTIR Microscopy, Photoacoustic step-scan and Raman microanalysis techniques have been used for years to characterize these materials. This paper will explore the benefits and weaknesses of these methods including the hardware and software tools needed. Guidance on which technique is the most appropriate for different analysis scenarios will be presented.

#### Keywords:
Food Safety, FTIR, Molecular Spectroscopy, Pharmaceutical

#### Application Code:
Food Safety

#### Methodology Code:
Molecular Spectroscopy
Poly-L-lactide ( PLLA) is a biocompatible, biodegradable, semi-crystalline and optically active polymer that is widely used as an implant and artificial cell scaffold material. The degree of crystalline character of PLLA influences its electromechanical properties such as elastic modulus and piezoelectric character. From its amorphous state, PLLA can be made more crystalline by drawing the polymer at a temperature above the glass transition, but below its crystallization temperature. Cold-drawing the PLLA to higher draw ratios increases its piezoelectricity and rigidity, two important considerations in the development of improved implants and biodegradable matrices. In the work presented here, a non-destructive and non-contact optical method for characterizing tailored PLLA substrates using Raman imaging and Raman polarization microscopy is described. The method utilizes a novel chemometric method to generate image contrast based on the degree of crystalline character. The image results enable a preliminary understanding of differences in bond orientations across the spatial extents of the sample and differentiated sample regions that are more crystalline from those that are more amorphous. A complete description of the optical method is presented along with a theoretical description of the chemometric technique.
The possibility of large enhancements in the radiative rates of emitters placed inside a cavity has been known since the middle of the last century (E.M. Purcell, Phys. Rev. 69, 681). We have recently shown that very large Purcell enhancements can also be achieved for Raman processes when high-finesse Fabry-Perot microcavities are employed. In the case of Purcell enhanced Raman scattering (PERS), the spontaneous Raman emission rate is inversely proportional to the cavity mode volume. In the case of Purcell enhanced CARS (PECARS), the anti-Stokes emission rate is inversely proportional to the square of the cavity mode volume.

The PERS effect was demonstrated for the 1,388 cm\(^{-1}\) Raman transition of CO\(_2\) in CO\(_2\)-air mixtures in a high-finesse (50,000) microcavity having mirrors with 40 um radius of curvature and cavity length near 30 um. The cavity length was adjusted to be resonant with both the pump laser wavelength near 867 nm and the Stokes wavelength near 985 nm. Under these conditions, the Raman emission from the cavity was found to be 2\times10^7 stronger than that estimated for free space. Approximately a factor of 3,000 increase was from the intracavity build-up of the pump laser power, and another factor of 7,000 attributable to the Purcell enhancement.

Experiments were also performed to investigate PECARS on the same CO\(_2\) Raman transition. The results show that similarly large Purcell enhancements should be possible for the anti-Stokes emission. The implications of PERS and PECARS as new spectroscopic techniques, including the possibility of single molecule detection, will be discussed.
The use of a coupled Rheometer and Raman spectrometer for obtaining comprehensive insight into a materials behavior is presented. Rheology is the analytical method of choice to correlate the absolute flow and deformation characteristics of a given product with its behavior towards a certain processing or application step. However, Rheology as an integral method only yields answers on the bulk of the investigated sample towards certain absolute shear or deformation conditions. It does not give any insights into what is actually happening on the molecular level during a certain processing step.

Raman spectroscopy has shown its ability as a powerful, effective and non-invasive method for chemical analysis. Coupling a Rheometer with a Raman spectrometer provides direct information about the molecular reaction kinetics and the mechanical properties. This is extremely useful for studying the crystallization behavior of polymer melts during processing. It can also provide insight for in-situ characterization and monitoring which can be challenging when working with on-line techniques as only relative flow fields are characterized.

Finally, the coupling gives the researcher the advantage of maximizing and synchronizing the information gathered from a single measurement as well as enabling transient information on their materials during fast processing conditions.

In this contribution we present results obtained with the brand new combination of a Thermo Scientific HAAKE MARS rheometer with a Thermo Scientific DXR spectrometer. Details of this unique new set-up as well as selected results including shear and temperature induced crystallization will be presented.
Heavy metals and associated compounds are potentially the most toxic elements for the environment. Generally, these metals are very toxic for living organisms, being dangerous to human health when present in drinking water and food. Various biological effects of heavy metals have been described; at the molecular level they decrease the activity of certain enzymes. The mechanism of enzyme inhibition by heavy metals is based on the interaction of metal ions with exposed thiol- or methylthiol groups of protein amino acids, often forming the active site of enzyme. Mercury is a very harmful pollution compound because of its great volatility and of its possibility to easily combine with many organic compounds, resulting in organomercury derivatives. These compounds are toxic and able to concentrate in living bodies, especially fishes, which become improper for human consuming. A fast spectrometric kinetic method for mercury(II) ions determination based on their property to inhibit glucose-oxidase activity is presented. The measurement of enzyme activity was based on enzymatic reduction of benzoquinone to hydroquinone and on the measurements of the initial rate of hydroquinone absorbance increase at 290 nm. A calibration curve for Hg(II) concentrations was obtained in the 0.05–1.0 µg/mL range, with a detection limit of 0.015 µg/mL and a relative standard deviation of 2.7%. The analysis time was 2 min. Determination of mercury in a real sample was in good agreement with the declared mercury concentration.
Abstract Text
The measurement of Airborne Molecular Contamination (AMC) in clean rooms requires highly sensitive detection methods as typical AMC amount fractions are at nmol/mol level and below. In the last decades cavity based spectroscopic methods such as CRDS and CEAS have been introduced which can reach such level of sensitivity. However the number of species that can be detected with a single instrument is inherently limited by the cavity mirrors. Within the EMRP project MetAMC (Metrology for Airborne Molecular Contamination) VSL develops an instrument which is suitable to detect a large number of species yet at high sensitivity. The instrument is based on the well-known photoacoustic technique. The required sensitivity is obtained by using a high power, yet compact mid-infrared light source developed by Taiwanese partner. To handle reactive and adsorbing AMCs the measurement cell is designed of glass and a special sampling system has been constructed. The instrument will be discussed in detail and initial results will be presented.

Keywords: Materials Science, Photoacoustic, Specialty Gas Analysis
Application Code: Quality/QA/QC
Methodology Code: Molecular Spectroscopy
Nickel Carbonyl and Iron Carbonyl are highly toxic vapors and pose a human health hazard at concentrations as low as several PPB in air. This paper will present an FTIR based sensor for Nickel Carbonyl and Iron Carbonyl that achieves a limit of detection of these components below 1 PPB. Furthermore, the FTIR is not confused by interferences that often lead to false alarms with conventional chemiluminescent sensors. Components like Nickel and Iron Carbonyl when released in air are often accompanied by significant concentrations of Carbon Monoxide which is also measured by the FTIR. The FTIR based sensor does not require any consumables, calibration gases or zero and span verification. Since Nickel and Iron Carbonyl are so highly toxic, a high reliability combined with effective diagnostics that will indicate the occurrence of malfunction is essential.
### Abstract Text

Some endocrine disruptors, such as active substances of some medicines, are known to be concentrating in the environment due to the inefficiency of conventional wastewater treatments to remove these residues. The catalytic photodegradation of these contaminants is a promising solution to the elimination of these compounds but requires further optimisation. The new technologies are first assessed in the laboratory for some pollutants or photodegradation markers and, if proved efficient, applied to more complex systems.

This work presents a strategy to develop reliable detailed models of the quantification of the efficiency gain of the catalytic photodegradation of methylene blue. Methylene blue was chosen since it is a very popular compound for assessing and comparing the efficiency of photocatalytic degradation processes. These models are used to report the photocatalytic gain with uncertainty to allow the comparison of results of different tests performed in the same or different laboratories, and to minimise determination uncertainty. The uncertainty was evaluated using the so called “bottom-up” approach. The methylene blue was determined by UV/Vis spectrometry at various wavelengths. To reach a relative standard uncertainty of less than 2 \%, concentration of methylene blue after photodegradation larger than 5 mg L\(^{-1}\) need to be determined. This uncertainty allows the reliable determination of variations of efficiency gain larger than 8.4\% [1].

This work was supported by Fundação para a Ciência e Tecnologia (FCT) under project PEst-OE/QUI/UI0612/2014 and PEst-OE/QUI/UI0536/2014.

### Keywords:
- Method Development, Process Control, Quality, UV-VIS Absorbance/Luminescence
- Quality/QA/QC
- Molecular Spectroscopy
New Products at Pittcon 2015

High Speed WDS Spectrometer for SEM Provides Rapid Id of Overlapped Peaks from Minor and Trace Constituents (WDS) / Materials

Energy Dispersive X-ray Spectroscopy (EDS) is the easiest and fastest way to perform x-ray spectroscopy in an SEM. However, several x-ray line overlaps are too close to resolve by this technique. This is particularly true if one or more elements are present in low amounts or if only low energy lines are available. Wavelength Dispersive X-ray Spectroscopy (WDS) provides much higher resolution and can easily resolve most overlaps but traditional WDS instruments require well trained, dedicated operators and are relatively slow.

A novel WD spectrometer has been designed which brings the benefits of WDS to the typical SEM/EDS laboratory. The use of direct drive motors with no gears belts or pulleys make it extremely fast and precise. It can change focus from one x-ray line to another in under a quarter second. No utilities are needed for operation besides electricity. It eases the need for training by automating most of the decisions normally made by the operator. A hybrid x-ray optic focuses x-rays onto the resolving crystal providing improved soft x-ray sensitivity and greatly reducing size restraints on the SEM mounting port. The result is a very fast WD spectrometer that can attach to most SEMs and is almost as easy to use as EDS.

The typical SEM operator can use this device to apply the high resolving power of WDS to peak identification during EDS analysis obtaining energy scans in just a few minutes which greatly amplifies the abilities of the SEM/EDS laboratory.

Keywords: Elemental Analysis, Instrumentation, Materials Characterization, Microscopy
Application Code: Materials Science
Methodology Code: Surface Analysis/Imaging
Over the last decade, considerable attention has been given for microchip electrophoresis in analytical science because of its many advantages including feasibility of miniaturization, minimal sample/solvent usage and speed. As part of a senior level problem-based learning analytical course, an assay that involved microchip electrophoresis (ME) was developed by undergraduate students. The problem that was assigned is as follows: both natural and synthetic antioxidant supplements are becoming popular among human beings because of their ability to retard the activity of pro-oxidants. A newly established company is seeking for a fast, cost effective assay, which can be performed without a highly equipped conventional laboratory. After a comprehensive literature search, the students chose microchip electrophoresis with amperometric detection for assay development. The assay focused on the most commonly consumed antioxidants: ascorbic acid, cysteine and glutathione. Two commercially available antioxidant supplements were used to demonstrate the capability of the method. Separation was carried on PDMS microchips using a 10 mM phosphate buffer containing 0.5 mM SDS as the surfactant at pH of 7.4. Detection was accomplished amperometrically at a Pt electrode. Calibration plots were prepared. The calculated antioxidant concentrations were then compared with the results obtained with LC-UV. Substantially reduced analysis times were obtained with ME relative to LC.
According to the regulations and guidelines set by the U.S. Food and Drug Administration (US FDA), the European Medicines Agency (EMA) and other regulatory agencies, extractable and leachable information must be included in applications for medical devices and container closure systems packaging human drugs and biologics. The Product Quality Research Institute (PQRI) Leachables and Extractables Working Group have recommended incorporation of multiple extraction techniques that employ vigorous extractions using multiple solvents of varying polarity in Controlled Extraction Studies. However, traditional Soxhlet or reflux techniques recommended by PQRI are labor intensive (>24 h/sample) and consume large quantities of solvent (>150 mL/sample). The accelerated solvent extraction (ASE) technique is an automated extraction technique with many advantages, including more efficient extraction, reduced extraction time (<0.5 h/sample), reduced solvent use (<30 mL/sample), and more flexibility in solvent selection. It is a powerful technique to reliably extract compounds from polymeric materials.

In this study, direct comparison of Thermo Scientific™ Dionex™ ASE™ 350 and Soxhlet were conducted. A polypropylene pill bottle and a transdermal patch pouch were extracted with 2-propanol and n-hexane using the Thermo Scientific™ Dionex™ ASE 350 system and Soxhlet, respectively. The extracts were analyzed using a Thermo Scientific™ Dionex™ UltiMate 3000 LC system coupled with a Thermo Scientific Q Exactive™ Hybrid Quadrapole-Orbitrap™ Mass spectrometer.

Compared to Soxhlet, ASE delivers comparable or more efficient extractions using less time (<0.5 vs. >24 hr per sample) and less solvent (<30 vs. >150 mL per sample). Extractions using multiple solvents of varying polarity are necessary in Controlled Extraction Studies, because different solvents can provide additional information on extractables.
Understanding Separations in HILIC Chromatography: Impact of High Organic on Solute Descriptors

Interest in chromatography using aqueous-organic mobile phases high in organic content (aqueous normal-phase, ANP, HILIC) has continued to build in recent years.[1, 2] In this mode of chromatography analyte retention increases monotonically with an increase in the organic component of the mobile phase. In previous studies, significant contribution of stationary phase chemistry toward the manipulation of retention and selectivity in ANP has been demonstrated.[3] The aim of this continuing study was to further enhance our knowledge of retention mechanisms that dominate in this interesting and useful mode of chromatography.

Ion exchange has been shown to contribute significantly toward retention and selectivity in HILIC separations. Solute descriptors important for chromatographic processes involving ion-exchange such as ionization constants (pKa/pKb) are often only available as measured in aqueous environments. When operating in HILIC mode, one deviates significantly from the aqueous environment and thus from these aqueous-based descriptors. It is therefore important to better understand the ionization state of the analytes and stationary phases in the HILIC environment. In this study, the influence of high organic content on basic and acidic pKa values as well as mobile phase pH dependence is explored. The impact of the descriptor variation is related to real chromatographic separations providing enhanced understanding, prediction and control over HILIC separations.


Keywords: HPLC, Ion Exchange
Application Code: Pharmaceutical
Methodology Code: Other
Using Area-Under-the-Curve (AUC) as a Tool for Validating the Hypoglyceamic Effects of Sida acuta Ethanolic Leave Extract in Experimental Diabetes

Sida acuta, a tropical plant, is used in traditional medicine to control of diabetes. The heterogeneity of diabetes has placed a demand on the search for newer hypoglyceamics. Thus the hypoglyceamic effect of Sida acuta leave extract in alloxan-induced diabetes relative to a standard drug (metformin) was evaluated and the predictability of Area under the Curve (AUC) as a tool for monitoring the hypoglyceamic potential of hypoglyceamic agents established.

Diabetic rats with blood glucose levels (BGLs) > 200 mg/dl were screened for the study following a 72hrs post-alloxan induction (150mg/kg/i.p). The baseline BGLs were determined prior to S. acuta extract and metformin treatment. Treatment with extract and standard drug were administered as follows: group II (induced only); group III (induced + metformin 2.57mg/kg/p.o); group IV (induced + S. acuta 200mg/kg/p.o); group V (induced + S. acuta 400mg/kg/p.o); group VI (induced + metformin + S. acuta 200mg/kg/p.o) and group VII (induced + metformin + S. acuta 400mg/kg/p.o).

BGLs were observed at 0hr, 1hr, 3hr, 6hr, 24hr, 48hr, 72hr, 7days, 14days and 21days. Statistical analyses were carried out using graphpad prism 5.0. Mean BGLs were compared using the student paired t-test and ANOVA (p<0.01) and the AUCs determined from pharmacokinetic plots.

Relative to baseline and control groups, a significant decrease in BGLs was observed (p<0.001) in the treatment groups. The percentage decrease in BGLs trends as S. acuta 200mg/kg (86.25%) > metformin (80.90%) > S. acuta 400mg/kg (73.50%) > metformin + S. acuta 200mg/kg (54%) > metformin + S. acuta 400mg/kg (41.90%). The % reduction in AUC followed the same trend, thus establishing evidence of reduced glucose in the physiological milieu.

Sida acuta (200mg/kg) showed a better hypoglyceamic property than Metformin. The AUC best correlated with hypoglyceamia and thus can be used to monitor BGLs and validate the significant anti-diabetic potentials of Sida acuta ethanolic extract.

Keywords: Analysis, Biological Samples, Data Analysis, Drugs
Application Code: Pharmaceutical
Methodology Code: Other
Effects of Sample Clean-Up and Use of High Purity Additives in Minimizing Ion Suppression in UHPLC/LC-MS Applications

UHPLC using UV or MS detection is now a routine technique in most analytical laboratories. The development of instruments and LC columns in the recent years improved the performance of fast and highly resolved separations significantly. Fused Core and sub 2 micron particle columns now enable the full performance of a UHPLC system with a maximum of sensitivity and chromatographic efficiency. Mobile phase impurities and the ion suppression effect are still pose concerns for the most common ESI source.

The results presented in this poster show the positive effect of a sample clean-up step prior to LC-MS analysis. Complex sample matrices, such as food samples, often cause ion suppression by co-eluting components, such as sugars or phospholipids. As, demonstrated in this poster, this issue can be resolved by using high purity additives.

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Abstract Text

Keywords: Liquid Chromatography/Mass Spectroscopy, SPME, Titration
Application Code: Pharmaceutical
Methodology Code: Other
Effect of Meglumine-Stearate Supramolecular Polymer Hydrogel Against C. Albicans Biofilm

Candida albicans is a major human fungal pathogen, causing a variety of infections, ranging from superficial to life-threatening [1]. Recent evidences suggest that the majority of disease produced by this pathogen is associated with biofilms growth. Microbial biofilms can be defined as communities of surface-attached microbial cells firmly encased in a layer of extracellular matrix (ECM). The biofilm lifestyle confers resistance to antimicrobials, and prevents access by host inflammatory cells and can also alter host metabolism [2,3]. Currently, wide ranges of anti-biofilm strategies have emerged, their focus include increase the permeabilization of biofilm in order to antimicrobials can penetrate deeper of this microbial community [3]. This study has evaluated the effectiveness of a supramolecular polymer together with the antifungal fluconazole against C. albicans biofilm. Supramolecular polymer hydrogel were derived from stearic acid and the aminocarbohydrate meglumine that self-assembled in aqueous phase through hydrophobic interactions and hydrogen bonds. This supramolecular gel can disrupt the ECM that is considerate a principal resistance mechanism of biofilms, once hamper the antimicrobial penetration. The incubation (24 hours) of mature C. albicans biofilms with the polymer (4%) associated with fluconazole (65 µg mL-1) promoted ~ 70% inactivation of these fungi, while only the antifungal at same conditions inhibited ~ 40% of C. albicans.

References:

Keywords: Biopharmaceutical, Pharmaceutical, Polymers & Plastics, Thermal Analysis
Application Code: Pharmaceutical
Methodology Code: Other
Due to the high cost and time frame associated with drug development, methods for improving efficiency while simultaneously reducing overall expenditure are critical. A specific area focused upon for the reduction of materials is in vitro pharmacokinetic (IVPK) models. The goal of such models is not to completely eliminate animal models but to work in tandem to eliminate costly animal studies by utilizing IVPK models. Current IVPK models, such as the hollow fiber chamber reactor, are able to successfully mimic in vivo PK curves but at a large cost due to large quantities of media and synthesized drug. Here, microfluidics are employed, due to the reduced volumes, i.e., milliliter or microliter, to investigate the potential of developing an IVPK device. Initially PDMS, a staple of the microfluidic community for decades, was explored as a platform for the IVPK model. While the absorbance (Cmax of 2 M) and elimination (half-lives of 0.5, 2, 4, and 8 hours) portions of the curve could be observed, the two could not be monitored on the same device due to the fragility of the membrane separating the channel from the well after flow times of 8 hours. PK curves were also lacking reproducibility owing to the single use nature of the PDMS devices. A 3D-printed platform resulted in a rugged reusable device, which is amenable to such automated infrastructure as plate readers and auto samplers. The reproducible curves produced with the 3D-printed device allowed for a mathematical prediction model for both the half-life and Cmax.

Keywords: Biopharmaceutical, Lab-on-a-Chip/Microfluidics, Liquid Chromatography/Mass Spectroscopy
Application Code: Pharmaceutical
Methodology Code: Microfluidics/Lab-on-a-Chip
# Session # 1230  Abstract # 1230-17

**Session Title**: Pharmaceutical - Vibration Spec, Molecular Spec, Others  
**Abstract Title**: USP Monograph Modernization

**Primary Author**: Leonel M. Santos  
United States Pharmacopeia

**Co-Author(s)**: Ramanujam S. Prasad

**Date**: Tuesday, March 10, 2015 - Morning  
**Room**: Exposition Floor, Hall F, Aisles 390

**Abstract Text**

The modernization of official monographs continues to be a key initiative at USP after the formal implementation in 2013. The key benefit of the modernization scheme is the evaluation of the identity, strength, and purity of drugs. A summary of the accomplishments, current status and future direction of monograph modernization will be presented. Important issues and challenges affecting modernization of monographs as well as strategies to include some over-the-counter (OTC) drug monographs will be presented and discussed.

**Keywords**: Analysis, Drugs, Pharmaceutical, Reference Material  
**Application Code**: Pharmaceutical  
**Methodology Code**: Chemical Methods
Estrogens are classically female reproductive hormones while they also regulate reproductive processes in males. The effects of estrogens are mediated by estrogen receptor (ER), which belongs to the superfamily of nuclear receptor, and it contains ERα and ERβ two subtypes. Since ERs have been found to be related to many different diseases, including osteoporosis and breast cancer, they have drawn a lot of attentions by medicinal chemists in the past decades. Thus selective estrogen receptor modulators (SERMs) have been developed, which are widely used in clinical for the regulation of ERs in different tissues, such as Tamoxifene (1) and Raloxifene (2). Since the existing SERMs show some serious side effects, a lot of different core structure ligands were synthesized and evaluated, e.g., pyrazoles (3), furans (4), pyrroles (5), and even more complex benzo[b]oxepines (6) and so on. These compounds showed good to excellent binding affinities to ERs depending on different substituents on the core, and some ligands possess certain subtype selectivity.

As a sustaining interest in developing novel core structure compounds as ER ligands in this presentation, we will report our latest efforts to develop a novel series of phthalaz-(2H)-one core derivatives as ligands for estrogen receptor. The binding affinities of these compounds are moderate and most of them were selective for ERβ. Reporter gene assays reflect that they are ERα agonist and ERβ antagonist. Molecular modeling suggests these ligands have a similar binding pattern as estradiol, which might be responsible for their agonism.

References
Sustained releases tablets of Diltiazem hydrochloride were formulated by employing hydroxypropyl methylcellulose (HPMC K100 M) and the sustained release behaviour of the fabricated tablets was investigated. Sustained release matrix tablets containing 120 mg Diltiazem hydrochloride were developed using different drug: polymer (HPMC K100 M) ratios. Tablets were prepared by wet granulation technique. Formulation was optimized on the basis of acceptable tablet properties and in vitro drug release. The resulting formulation produced robust tablets with optimum hardness, consistent weight uniformity and low friability. All tablets but one exhibited gradual and near-complete sustained release for Diltiazem hydrochloride (96-100%) at the end of 24 h. The results of dissolution studies indicated that formulation B5 (drug to polymer 1:1.25) was found to be most successful as it exhibits drug release pattern very close to theoretical release profile. A decrease in release kinetics of the drug was observed on increasing polymer ratio.
Laser Induced Breakdown Spectroscopy (LIBS) is an ideal analytical technique for in situ analysis of any elemental composition. In this work, we have performed a comparative study of the quantitative and qualitative analysis of atomic and molecular emission from LIBS spectra. In our experiments, a mixture of SrCl2 and Al2O3 in powder form was used as sample. The atomic emission from Sr and molecular emission from SrCl2 and SrO observed in LIBS spectra were analyzed. The optimum laser energy, gate delay, gate width for selected atomic lines and molecular bands are determined from the spectra recorded at various experimental parameters. The optimum experimental conditions were used to collect the calibration data for the selected atomic and molecular emission spectra and use to determine the limit of detection (LOD) of Sr. The details of the results of these measurements will be presented.
Recently, the photoacoustic effect has been utilized to allow 3D imaging in turbid media (e.g. living tissue). To date, most work has used hemoglobin as an endogenous agent for biomedical imaging, though some work has been done in developing exogenous contrast agents. However, few chemical reporter molecules have been identified for conducting imaging based on local properties (e.g. pH, temperature). We present the development of a pH reporter molecule for measurements in turbid media, including living tissue, and focus on the properties required to permit well-behaved chemical measurements.
The present study was carried out to investigate the antibacterial activities of 2-aminophenol Schiff bases and metal complexes. The increasing microbial resistance to existing antiseptics and disinfectants has necessitated the search for new compounds with potential effects against pathogenic organisms. Both developed and resource-poor countries are faced with the burden of healthcare-associated infections. The risk of infection with multidrug-resistant organisms can be overcome with the use of disinfectants in homes, veterinary and hospital environments. There is therefore a need to synthesize and investigate new antiseptics and disinfectants on a range of microorganisms. In line with this, novel copper(II) complexes with Schiff bases derived from substituted benzaldehydes and 2-aminophenol have been synthesized using condensation method.

The compounds were fully characterized using elemental analysis, atomic absorption spectroscopy, infrared spectroscopy, [sup][1/sup]H NMR, electronic absorption spectroscopy and thermal gravimetry analysis. The Schiff bases and their metal complexes were screened for [i]in-vitro[/i] antibacterial activities against 6 human pathogenic bacteria; [i]Escherichia coli (ATCC 8739)[/i], [i]Staphylococcus aureus (ATCC 6538)[/i], [i]Pseudomonas aeruginosa (ATCC 19582)[/i], [i]Bacillus cereus (10702)[/i], [i]Enterococcus faecalis (ATCC 29212)[/i] and [i]Kribesella pneumonia (ATCC 10031)[/i]. Sodium hypochlorite was used as a reference compound. The result showed that Schiff bases exhibited moderate inhibitory activity against the tested microorganisms similar to sodium hypochlorite. The Schiff base metal complexes exhibited higher antibacterial activity compared to sodium hypochlorite. Our results show that these complexes can be employed as active ingredients in development of broad spectrum antiseptic agents.

The authors acknowledge support from National Research Foundation, University of Zululand, South Africa

Keywords: Atomic Absorption, Elemental Analysis, NMR, UV-VIS Absorbance/Luminescence
Application Code: Drug Discovery
Methodology Code: Chemical Methods
A series of Benzimidazole derivatives synthesized were found to exhibit excellent antimicrobial properties. The formation of bioactive heterocyclic analogues was confirmed using IR spectrophotometer (Bruker alpha-t). The titled heterocyclic analogues were formed by condensation of two intermediates, which was confirmed using vibrational frequencies of the functional group(s). Thus Vibrational Spectroscopy plays a vital role in determining the formation of drug like molecules and thus can be used as a tool for determining the structure in drug discovery process.

Keywords: Drug Discovery, Vibrational Spectroscopy
Application Code: Drug Discovery
Methodology Code: Vibrational Spectroscopy
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**Abstract Text**

Optofluidics is a scientific instrumentation manufacturer that makes systems for analyzing small scale particles in solution. Our technology enables single submicrometer particle Raman spectroscopy, to chemically identify and characterize small particles in solution with applications to biopharm quality control. In addition, it also enables surface analysis of single submicrometer particles to determine surface functionalization success and nanoparticle coating analysis/stability, with applications to nanomedicine and nanoparticle formulation. Unlike traditional light scattering and spectroscopy systems, we use chip based optics to take on the challenge of transcend particle analysis beyond the traditional "sizing" paradigm.

**Keywords:** Biotechnology, Molecular Spectroscopy, Nanotechnology, Particle Size and Distribution

**Application Code:** Bioanalytical

**Methodology Code:** Molecular Spectroscopy
Ketoconazole (KTZ) is a highly effective broad spectrum antifungal agent. Several methods for the assay of KTZ have been reported, including potentiometric methods that are approved standard methods in the British Pharmacopeia (BP) and United States Pharmacopeia (USP). Intensive investigations were performed to assay KTZ using different spectrophotometric methods. Recently, EPR spectroscopic analytical methods have been used to monitor radical formation through the chemical oxidation of KTZ in the presence of Cerium(IV) in acid media. The method was shown to be precise, accurate, specific, and fast for quantitatively determining the KTZ concentration in tablet or cream formulations. This method is precise, fast, and consumed less reagents compared to the other reported methods. However, special sample preparation and large quantity of KTZ are required. In the present study, a new inexpensive two-electrode cell for use in in-situ electrochemical-electron paramagnetic resonance (EC-EPR) spectroscopy techniques was developed and utilized for the analytical determination of Antifungal drug ketoconazole (KTZ). A schematic illustration of the EC-EPR setup is given below. The developed method relied on monitoring the peak-to-peak EPR signal intensities obtained from KTZ-radical species that were generated electrochemically at disposable graphite pencil electrode (GPE) surfaces. Optimization of the EC-EPR parameters enabled KTZ radical detection at a concentration and with a volume that were one order of magnitude lower than the corresponding concentrations and volumes tested using chemical oxidation analysis techniques.
Propolis is a natural and sticky, resinous, material collected by bees from different botanical materials and exudates found on plant wounds, mixed with own substances from mandibular and hipofaryngeal secretions, and transformed in order to be used for sealing different holes in the hive, to clean the comb cells or to hygienist hives. The studies of propolis are usually associated with the identification and determination of their biogeographic origin. Usually instrumental analysis is necessary for evaluations and characterization of this natural product. The object of this research has been focused on UV-Vis and infrared spectroscopy as a tool for classification of Colombian propolis from different biogeographical zones.

Twenty-three samples of crude propolis were extracted with ethanol 96º, determining the performance in the soft extract in each removal. The infrared and UV-VIS spectroscopy spectrum were measured and registered. Organoleptic properties and spectral properties were used in for the characterization and classification of the types of Colombian própolis. Propolis spectra with 320 nm have total flavonoid content low tan 2 g /100 g. Propolis spectra having a plateau as UV maxima between 320 and 395 nm exhibit a total flavonoid content approximately of 5 g/100 g; spectra samples having 295 nm, show total flavonoid between 5 and 10 g/100 g. IR (cm-1) spectra show signals for C–H bonds of aromatic compounds, in the region of 3300–2700 range, carbonyl group, (C=O) with a strong absorption in (1820–1660); O–H band group found at (3300–2500); C=C band was as a weak absorption (1650); Aromatic ring as general gave rise weak absorption in (1650–1450). Additional procedures for classification demand the use of chemometric analysis.
Ofloxacin is a synthetic chemotherapeutic agent used to treat severe and life threatening infections. It is a fluoroquinolone antibiotic with broad spectrum of activity. This study aimed at evaluating some physiochemical parameters and quantitative quality of ten brands of ofloxacin tablets randomly obtained from some pharmaceutical stores in Mushin Area, Lagos state.

The physicochemical tests carried out include uniformity of weight, hardness, friability, disintegration and dissolution while the Ultraviolet/Visible spectrophotometric method was adopted for the quantitative analysis. The dissolution rate and disintegration time were determined in simulated gastric fluid (SGF). Similarity factor ($f_2$) was used to assess bioequivalent between the innovator brand and the other brands.

The results indicated that all the ofloxacin brands complied with the official specification for uniformity of weight, friability, and disintegration. However, the dissolution profiles in 0.1N HCl (SGF) showed that brand F failed to attain 80% dissolution in 30 min, despite the fact that it passed the quantitative test. Brands F, G and J has similarity factor less than 50% compared to the innovator brand (brand A). This shows that brands F,G and J are not similar with the innovator brand. The UV spectrophotometric assay of ofloxacin tablets revealed that two brands (B and G) contained less than 90% of labeled chemical content. This suggests that chemical equivalence does not indicate bioequivalence.

In conclusion, 100% of the samples passed the physical tests while 80% of the samples passed the quantitative assay and 70% passed the similarity test.
Drug stability studies, including chemical, physical, and microbiological stability, is mandatory for development of pharmaceutical active ingredients and products. No significant changes (e.g. appearances, drug contents, impurities, polymorphisms, sterility, etc.), which impact pharmaceutical quality, safety and efficacy, should be observed during the drug shelf-life. Methods for stability studies must be stability-indicating to enable the determination of drug substances and the quantitation of degradation products upon exposure to various stress conditions (e.g. thermal stress, hydrolysis (acid, alkaline, neutral), photolysis and oxidation).

Two stability-indicating capillary electrophoretic (CE) methods were developed for the stability study of anti-diabetic drugs, metformin (MET) and gliclazide (GCZ). For MET and its major degrade (CGN), capillary zone electrophoresis (CZE) was proposed in 40 mM citrate buffer (pH 6.7) using a fused-silica capillary with an effective length of 60 cm and an inner diameter of 50 µm, injection at 50 mbar for 5 s at 30°C with a voltage of 15 kV and photodiode array detection (PAD) at 214 nm. For, GCZ and its impurities (gliclazide impurities B (GZB) and F (GZF)) were separated using 10 mM phosphate buffer (pH 7.0) containing 15 mM sodium dodecyl sulfate on a fused-silica capillary with an effective length of 40 cm and an inner diameter of 50 µm, injection at 50 mbar for 5 s, temperature of 25°C, a voltage of 20 kV and PAD at 225 nm. Both methods were validated and applied to establish stability profiles, degradation kinetics (e.g. rate constants, half- and shelf-lives) and pathways of both drugs.

Keywords: Capillary Electrophoresis, Drugs, Quality Control
Application Code: Pharmaceutical
Methodology Code: Capillary Electrophoresis
Abstract Text
To determine the optimal interactions between flavonoid ligands and Benzodiazepine Receptor (BzR) on GABAA, 4D-QSAR analysis was applied using CoMCET method. The electronic properties were used as the 3D descriptors created by ETM. GA (genetic algorithm) was performed to analyze these descriptors roles in all activities and to construct a model that would be able to predict binding affinity for a wide range of molecular structures. Model validation was carried out by applying LOO-CV in addition to external validation. Using a Pha model together with five partial atomic charges as descriptors, a QSAR model of nonlinear equation was established, then the Q2 and R2 possessing fairly high values of 0.806 and 0.761, respectively were calculated. The analysis of docking suggested good theoretical affinity and pharmacophoric points of the new compounds to the enzyme GABAARs/BZRs. Docking analysis was in harmony with the QSAR analysis.

Acknowledgement: work has been supported by The Scientific and Technological Research Council of Turkey (TUBITAK) Project no: 108T148.
Memantine, amantadine and rimantadine are amantadane drugs that act as an N-methyl-D-aspartate antagonist. Memantine and amantadine are neuroprotective agents, which are used for treatments of Alzheimer’s and Parkinson’s disease, respectively. Additionally, amantadine and rimantadine are antiviral drugs that are used in combination with oseltamivir for treatment of influenza. Analysis of these drugs can be problematic due to the lack of chromophores or fluorophores in their structures. Although several analytical methods have been proposed, including liquid chromatography-mass spectrometry (LC-MS), spectrofluorometry and gas chromatography-MS (GC-MS), simpler, faster and more cost-effective methods are still required. This study aimed to develop a fully automated procedure for the analysis of the drugs using in-line derivatization capillary electrophoresis with photometric detection. Standard solutions of the drugs were directly injected into the capillary then were in-capillary derivatised with a short plug of fluorescence tag fluorescamine, which was injected before the sample. Separation was carried out in 150 mM borate buffer (pH 9.5) using a 50 µm i.d. capillary with an effective length of 50 cm, with a voltage of 25 kV, temperature of 25[degree]C and the detection wavelength at 305 nm. The fluorescent product migrate at 5.3 min and was baseline separated from interference. The product formation increased linearly (r² > 0.98) to the amount of the analyte. Simultaneous separation of all products and application for real samples are under investigation. The method offers advantages in term of short analysis time and a low sample and chemical consumption, which can be beneficial to pharmaceutical analysis.
USP <232> and <2322> – What is the Sum of Toxic Elements That You Might Ingest Every Day?

In April, 2012 the United States Pharmacopoeia (USP) proposed that Chapter 231 be replaced with two new Chapters which outline ICP-OES/ICP-MS methods to determine a group of metallic contaminants in pharmaceutical products and supplements. The methods that have been summarized in General Chapters <232>, <233> and <2322> are undergoing final revision with a proposed implementation in December, 2015. Chapter <232> specifies the list of elements and their toxicity – Personal Daily Exposure (PDE), and outlines the method of analysis per maximum daily doses of the drug within specific drug delivery categories – oral, parenteral (intravenous injection), inhalation and large volume parenteral. The objective of the chapter <2322> is to limit the amounts of four of the most toxic potential elemental contaminants in finished dietary supplements: arsenic, cadmium, lead, and mercury (the “Big Four”). Many papers and articles have presented data from analysis of a single specific drug or supplement (based on the recommended dosage) using the new USP Method. However, it is common for people to take several medications and supplements daily. This poster presents data from an experiment to sum the USP 232 elements found in a hypothetical mix of a few common drugs and popular supplements that one person might take daily. It utilizes the tools outlined in USP Chapter 233 – sample preparation by closed vessel (microwave) digestion and analysis by ICP-MS.

Keywords: Consumer Products, Drugs, ICP-MS, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Enhanced Development of a Two-Step In-Vitro Dissolution Method for an Enteric Coated Formulation with the Aid of Micro-Dissolution Apparatus and UV-Fiber Optic Detection

An enteric-coated tablet formulation was developed for a BCS Class II compound with a layer of acid-resistant polymer coating to prevent drug release under acidic conditions. The active pharmaceutical ingredient (API) has poor aqueous solubility (<1 \( \mu \text{g/mL} \)) across a wide pH range. To develop the two-stage dissolution method as per USP <711>, we used a micro dissolution apparatus to perform solubility testing and screen different dissolution media. The in situ, real-time dissolution profiles were collected by a UV fiber optic spectrophotometer. After the dissolution media were selected, a Distek Symphony 7100 with dissolution apparatus I &II was employed to screen and optimize other dissolution method parameters. Among the several surfactants studied, SLS was selected since it results in good solubility of the API from pH 1.2 to 6.8, and provides satisfactory release of the core tablet. However, the direct application of SLS in acid media will cause slow dissolution of the enteric coating material. To solve this challenge, the final dissolution method was developed as a 2-tier method with media additions, with an initial acidic media stage, followed by a stage using pH 6.8 phosphate buffer. This dissolution method demonstrated that the enteric-coated tablet has minimal release under acidic conditions and full release at pH 6.8, which met the USP requirements for a delayed release formulation.
Adenosine-5'-triphosphate (ATP) supplements are a relatively new trend in the health and exercise industry, but the notion of a secondary source of energy outside of a normal diet is not new. It is well known that many of the dietary supplements on the market are unregulated. In fact the FDA estimates that up to 70% of supplement companies do not adhere to basic quality standards which can result in health complications. Due to the nature of the marketing taking place with respect to ATP supplements, it is important to determine the purity and accuracy of the ingredient labels because many of the ATP supplements also contain its hydrolysis products including adenosine-5'-diphosphate (ADP), adenosine-5'-monophosphate (AMP) and adenosine. Quantitative analysis of two commercial ATP supplements (Douglas labs and Hammer nutrition) was performed using capillary electrophoresis with UV detection. The four analytes were separated and detected using a run buffer consisting of 20 mM pH 7.48 phosphate buffer at 485V/cm field strength. It was discovered that each Douglas Labs ATP-20 tablet contained an average of 11.59% ATP, as well as 0.3% AMP and 0.2% ADP by weight. Adenosine was quantitated by microchip electrophoresis with electrochemical detection and was present at a concentration of 0.4% by microchip capillary electrophoresis. Hammer Nutrition PeakATP tablets contained a proprietary form of ATP that could not be quantitatively analyzed. P NMR spectra also confirmed the presence of ADP and AMP in both commercial supplements.

The Joint United Nations Program reported the estimate of people living with HIV was 33.4 million in the world in 2008[1]. Indinavir is a common drug used in HIV therapy. Recent studies have revealed the drug-drug interaction between venlafaxine immediate-release formulation and indinavir[2]. It was reported that interaction of racemic mixture of venlafaxine significantly decrease serum concentrations of indinavir by 28% thereby possibly lowering the therapeutic benefit of indinavir in HIV-treated patients. If a drug-drug interaction alters the balances of (-) and (+) enantiomers, it is possible that the other therapeutic actions of antidepressant such as efficiency in patients with chronic pain syndromes could be affected. The only way to rule out this type of drug-drug interactions is the assessment of the potential changes in (-) and (+) enantiomer serum concentrations of venlafaxine and its metabolites O-desmethylvenlafaxine, which is marketed as Pristiq®[3-4], and is about three times more potent than Vx in inhibiting norepinephrine(NE) presynaptic neuron reuptake[4].

In this study, a pharmacokinetic assay based on micellar electrokinetic chromatography-electrospray ionization-tandem mass spectrometry (MKEC-ESI-MS/MS) is under development to simultaneously separate the two enantiomers of both VX and ODVX. To achieve the simultaneous baseline enantiomeric separation of VX and ODVX in short analysis time, some MKEC parameters such as head groups of the surfactant and buffer pH etc. have been optimized. We are now at the stage of method validation and application to the patient plasma samples.

Reference

Keywords: Chiral, Chiral Separations, Mass Spectrometry, Solid Phase Extraction
Application Code: Pharmaceutical
Methodology Code: Capillary Electrophoresis
The development of medical physics in Russia recent years was associated with the attention of medics to physical characteristics of biological objects - tissues, blood, physiological liquids etc. The evaluation methods of biological objects structure - cells of human organism, bacteria, etc are developed. In this regard, there is a growing interest to devices and equipments to assess the physical characteristics of drugs, promising for use in the treatment of diseases. For example, the rheological characteristics of the skin medications are important. It is known that the standard methods of viscosimetry require a large volume of liquid and this is often inconvenient.

The possibility of application in dermatology the device constructed by Russian hematologists for the evaluation rheological properties of blood, suspension of erythrocytes and plasma are presented here. In this device the time of expiration of any biopolymer, viscous component or mixed drug solutions are registered automatically. Probe volumes of sample elastic - viscous biologically active fluid is equal to 250 microlitres. This is more economical than forehead methods for samples of the developed drugs in the treatment of skin. As the models of viscous drugs for skin were selected the water solutions of glycerin with concentrations ranging from 10 to 80 % of glycerin; these fluids comes in many pharmaceuticals. The series of porous membranes from polycaproamide, which are more sensitive to viscous properties of flowing liquids, were chosen as the models of the skin. The diameter of pores was 0.22 - 0.65 um, the surface structure of membranes was determined by atomic force microscopy. The results of obtained relations between the glycerin concentrations and the flow dynamics of viscous fluid through the porous films as skin model are presented.

Abstract Text

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Keywords:

Materials Characterization, Microscopy, Pharmaceutical, Rheology

Application Code:

Pharmaceutical

Methodology Code:

Physical Measurements
Hot Melt Extrusion (HME) has become increasingly adopted for the formulation of new chemical entities (NCE) to overcome solubility and dissolution rate issues. An important application of HME is the development of novel pharmaceutical applications such as co-extrudates. In which, a multi-layered extrudate is created by simultaneous hot-melt extrusion of two or more materials through a single die.

Raman imaging can be an excellent analytical technique to characterize the co-extrudates due to its sensitivity, speed, spatial resolution and highly detailed chemical information it can provide. In particular, the resulting images can be used to confirm the migration of the active pharmaceutical ingredient (API) between different layers.

Here we present the complete process of producing a co-extrudate in laboratory scale and applying Raman imaging technique to monitor the quality of the final product. Critical process steps and associated analytical parameters will be discussed in-depth.
The absorption and fluorescence behavior of a rigorously purified phenylalanine ionic liquid, L-Phenylalanine ethyl ester bis(perfluoroethanesulfonyl)imide are studied in the neat condition. The ionic liquid was prepared using a metathesis reaction between lithium bis-(perfluoroethylsulfonyl) imide (LiBETi) and L-Phenylalanine ethyl ester hydrochloride. The ionic liquid was obtained as a highly viscous and clear liquid at room temperature. The water content of the IL was determined using Karl Fischer titration. The possibility of racemization in the synthesized ionic liquid was evaluated using circular dichroism. NMR spectroscopy was used to determine its purity and its thermal properties were evaluated using thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC). The ionic liquid was found to be stable up to 292ºC with a melting point of 95ºC. The fluorescence emission of the neat CIL shifted to longer wavelengths as the excitation wavelength was increased. This is attributable to the existence of energetically different excited state species. The work involves studying the CIL’s viscosity and ability for column coating. It is used to analyze its ability to discriminate between various chiral analytes.

Keywords: Chiral, GC Columns, Thermal Analysis, UV-VIS Absorbance/Luminescence
Application Code: Pharmaceutical
Methodology Code: Thermal Analysis
Comparing Different Chemometric Techniques for the Determination of a Ternary Mixture of Betamethasone Dipropionate, Clotrimazole and Benzyl Alcohol

Different chemometric models were applied for the quantitative determination of betamethasone dipropionate (BMD), clotrimazole (CT) and benzyl alcohol (BA) in ternary mixture using their UV spectral data, namely, Partial Least Squares (PLS) and Artificial Neural Network (ANN). The effect of Genetic Algorithm (GA) as a variable selection tool was evaluated by comparing the results obtained from the PLS and ANN models with and without variables selection. The applied chemometric models were PLS, ANN, GA-PLS and GA-ANN. A calibration set composed 15 mixtures and a validation set composed of 12 mixtures were both constructed according to a 3-factor 5-level experimental design. The proposed methods were successfully used for the determination of BMD, CT and BA in pure powder form and in pharmaceutical preparation and the validity of the proposed methods was further assessed by applying the standard addition technique.

Keywords: Analysis, Chemometrics, Pharmaceutical, Spectrophotometry

Application Code: Pharmaceutical

Methodology Code: Chemometrics
The US EPA’s Tier 3 Ruling reduces allowed sulfur concentrations in gasoline from an average of 30 mg/kg to an average of 10 mg/kg, beginning in 2017. Refiners, importers and biofuel blenders of gasoline will be required to perform sulfur analysis at these ultra-low levels to report compliance throughout the supply chain. The Ruling also enacted use of a PBMS (Performance Based Measurement System) approach to allow reporting laboratories new flexibility regarding the sulfur test method they may use. Use of consensus group (e.g. ASTM) standardized test methods and mandatory quality control practices validate adequate measurement quality.

The objective of this project is to demonstrate that the recently revised ASTM D7220 sulfur test method initially used for EPA’s 2006 Diesel Ruling which reduced allowed sulfur concentration down to 15 PPM, can also be utilized to meet EPA’s Tier 3 Ruling. D7220 takes advantage of monochromatic EDXRF to reduce background radiation interference and produce a single energy of excitation for sulfur.

Experiments were conducted using a monochromatic EDXRF system with a close-coupled, doubly curved HOPG crystal to reflect Ag K\(^{\alpha}\) radiation, a vacuum chamber, a Pelletier-cooled Ag anode X-ray tube, and a Silicon Drift Detector. Sample volumes of 5 mL were analyzed. Testing parameters and example data that exhibit pass/fail conditions are described.

Findings conclude that compliance with all EPA PBMS requirements for both ultra-low sulfur diesel and gasoline for sulfur levels at and below 3 mg/kg, well within the Tier 3 Ruling, is demonstrated using the recently revised ASTM D7220 Method.

Keywords: Environmental, Fuels\Energy\Petrochemical, Sulfur, X-ray Fluorescence
Application Code: Fuels, Energy and Petrochemical
Methodology Code: X-ray Techniques
Aramar Experimental Center (AEC) is part of military organization of Brazilian Navy that develops the Nuclear Program for the first Brazilian Nuclear Submarine. It’s located at Iperó, São Paulo, in Sorocaba river basin, near a permanent conservation unit called Ipanema National Forest. The objective of this study is monitoring points upstream (A) and downstream (B, C) the effluent discharge of AEC (Fig.1a), using an alternative technique for direct analysis of total uranium and other metals, in sediment samples. The application of wavelength dispersion X-ray fluorescence (WDXRF) techniques with univariate calibration method and fundamental parameters, used for inter-elemental (or matrix) effects corrections with reduced sample preparation steps has shown promise for direct analysis of uranium, thorium, cobalt, lanthanum, cerium and lead. The assessment methodology performed by statistical tools using Certified Reference Material from International Atomic Energy Agency “Soil7”, showed acceptability of methods of analysis to agree with HORRA values, i.e. ratio of the observed relative standard deviation calculated from the actual performance data, to the corresponding predicted relative standard deviation calculated from the Horwitz equation, since the calculated values are <2 for all elements listed. The accuracy in terms a Z-score value has also been accepted, since for every element the values are <1.4, effectiveness proving of the proposed methodology. The quantification limits are between 0.1 and 2.3 [micro]g.g-1, enough to achieve the aims of this work. The analysis results (Fig.1b) showed there’s no significant influence after effluent discharge and nuclear area activities, when compared with a control point (D).

**Keywords:** Environmental Analysis, Monitoring, X-ray Fluorescence

**Application Code:** Nuclear

**Methodology Code:** X-ray Techniques
This paper evaluates the benefits of using borate fusion preparation combined with XRF to analyze chlorine in cement and raw material products. In this industry, it is important to quantify the chlorine content in the cement-related materials because the production process can be slowed down if the level of chlorine is too high. Also, the level of chlorine in most cement finished products must be maintained as low as possible because it reacts with the metal reinforcements in the structures. It reduces the strength and the lifetime of the concrete. Also, some types of cement need a specific amount of chlorine to give the product its distinct properties.

Many cement laboratories use an alternative method to XRF for their chlorine analysis since it is challenging to find CRMs with good certified values for chlorine. Integrating the analysis of chlorine into their borate fusion and XRF routines would allow them to reduce the number of analytical methods, diminish the workload and decrease the operations costs.

MATERIALS
All experiments were done with an electric fusion instrument: TheOx. LOI on all samples and CRMs were made on a Fisher Scientific Isotemp® muffle furnace. Spectrometer analytical conditions were selected and optimized to qualified standard method ASTM C 114 and to validate ISO/DIS 29581-2.

SIGNIFICANCE
This research examines the analysis of chlorine using a well-accepted analytical method for the analysis of all oxidized compounds found in cement related materials. The chlorine data compiled will be presented to explain how the chlorine calibration curve was elaborated and validated. The spectrometry, standards preparation and calibration strategies for chlorine will also be widely discussed in the presentation. Precision testing on the analysis of chlorine were done following the ASTM C 114 regulation for cement material analysis and accuracy evaluations were conducted following the standard methods ASTM C 114 and ISO/DIS 29581-2.

Keywords: Calibration, Quality Control, Sample Preparation, X-ray Fluorescence
Application Code: Materials Science
Methodology Code: X-ray Techniques
Abstract Text
Professor Redfield will talk on his early NMR work and then introduce field-cycling in a general way with mention of the good and not so good aspects of their recent approach.
Changes in the rate of recovery ($R_{1}$) of the phosphorus nuclear spins as a function of magnetic field (particularly in the region from 0.005 to 0.5 T) in the Redfield high resolution field cycling system provide a new way to explore phospholipid dynamics and interactions with amphitropic proteins. The methodology is particularly useful for identifying and localizing novel phospholipid binding sites on a protein that is spin-labeled on discrete sites. Whether the amphiphile is presented in a vesicle or micelle, or mixed with variety of other lipids, the differences in the proximity of the amphiphile to spin-labels can be easily monitored. Combining these NMR-derived $^{31}$P-electron distances with modeling provides testable models for discrete amphiphile binding sites. The method is illustrated with studies of three enzymes, transiently anchored on membranes, that are known to bind phosphatidylinositols but also have interactions with other phosphorylated amphiphiles (whereabouts unknown until this work). (1) A bacterial phosphatidylinositol (PI) specific phospholipase C forms a cation- lipid complex with phosphatidylcholine ~15 Å from the enzyme active site that enhances PI-specific phospholipase C binding to vesicles for processive catalysis. (2) The PH domain of Akt1 has a unique site for a cytotoxic alkylphospholipid near but not overlapping with the natural PI(3,4,5)P$_3$ binding site; the bound alkylphospholipid misorients the protein on the membrane thus preventing phosphorylation and kinetic activation. (3) The tumor suppressor PTEN has a binding site for its hydrolysis product PI(4,5)P$_2$ distinct from its active site whose occupation can aid in membrane binding and enhance enzyme catalytic efficiency.
The flexible nature of protein loops and the timescales of their dynamics are critical for many biologically important events at the molecular level, such as protein interaction and recognition processes. In order to obtain a predictive understanding of the dynamic properties of loops, we classify them into three types, namely fast loops with correlation times < 10 ns, slow loops with correlation times between 10 ns and 500 ns, and loops that are static over the course of the whole trajectory based on the detailed comparison of experimental and predicted NMR chemical shifts and order parameters from 500 ns MD trajectories of 38 different proteins. Chemical and biophysical loop descriptors, such as amino-acid sequence, average 3D structure, charge distribution, hydrophobicity, and local contacts were used to develop and parameterize the algorithm for the prediction of the flexibility and motional timescale of every protein loop. The results demonstrate that loop dynamics with their timescales can be increasingly well predicted, which helps better understand the various roles of protein loops for protein-protein interactions during biomolecular recognition events.
Ras proteins are critical regulators of multiple pathways involved in cellular growth control. Activating mutations in Ras genes are found in 33% of human cancers, with Ras well recognized as the most commonly mutated oncogene in human cancer. The population of activated, GTP-bound Ras in normal cells is controlled by protein regulators. Mutations in Ras associated with cancer promote cellular activation by populating the active state of Ras, most frequently by impairing the action of proteins that downregulate its activity. We are investigating novel mechanisms of Ras regulation by posttranslational modification of lysine and cysteine residues in the conserved guanine nucleotide binding domain. Our previous studies of K- and H- Ras have shown that monoubiquitination and cysteine oxidation can upregulate Ras activity and promote Ras-mediated tumorigenesis. We are further characterizing these and other posttranslational modifications. Multiple approaches are being used generate and characterize lysine and cysteine modifications, including mutagenesis, chemical biology and unnatural amino acid incorporation. To better characterize mechanisms by which these posttranslational modifications modulate Ras function, NMR, modeling, biochemical and biological studies are being conducted. Results from these analyses will be presented, along with our recent work on an oncogenic variant of Ras, K-Ras G12C, prevalent in lung cancer. We find that this activating mutation introduces a redox active cysteine that renders Ras G12C sensitive to thiol oxidation. Thiol oxidation at this site alters Ras activity in a manner distinct from other oncogenic mutations at position 12.

Keywords: Biospectroscopy, NMR, Protein
Application Code: Biomedical
Methodology Code: Magnetic Resonance
Pittsburgh Spectroscopy Award

**Structural Characterization of TRIM5 ▶ A Potent Restrictor of HIV Replication in Rhesus Monkeys**

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<th><strong>Primary Author</strong></th>
<th>Dmitri Ivanov</th>
<th>University of Texas Health Science Center</th>
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**Abstract Text**

TRIM5 ▶ retroviral restriction factor is a member of the TRIM family of RING E3 ubiquitin ligases. TRIM5 ▶ of the rhesus monkey binds to the HIV capsid and potently blocks HIV replication, whereas the human variant of the protein is not active against HIV. Affinity of TRIM5 ▶ for the capsid is a major host tropism determinant of HIV and other primate immunodeficiency viruses, but the exact molecular interface involved in this host-pathogen interaction and the details of the restriction mechanism are not fully understood. We use spectroscopic and biophysical methods to characterize the molecular basis of the TRIM5 ▶ -mediated antiretroviral immunity. NMR-based approaches have proven particularly powerful for this system. NMR techniques allow mapping of the TRIM5 ▶ capsid interactions mediated by the highly-variable and mobile loops of the C-terminal SPRY domain, whereas NMR studies of the mobility at the capsid-binding interface shed light on the remarkable ability of TRIM5 ▶ to restrict divergent retroviruses and to tolerate capsid mutations. In addition, NMR allows characterization of the weak, but critical, protein-protein interactions involved in the catalysis of poly-ubiquitin synthesis. Our data elucidate how SPRY-capsid interactions and the higher-order oligomerization of TRIM5 ▶ contribute to the activation of the E3 ubiquitin ligase function and HIV restriction. Our studies provide mechanistic insight into the emerging role of TRIM proteins in innate immunity.

**Keywords:** Biomedical, Biospectroscopy, Magnetic Resonance, Protein

**Application Code:** Biomedical

**Methodology Code:** Magnetic Resonance
Chemical recognition by complexation may occur in homogenous solution phase or in a separate phase that is immiscible with water. The former lays the foundation for molecular probes, complexing agents and indicators, the latter is at the heart of electrochemical detection and sensing. We will here show that nanomaterials allow us to blur the lines between the two previously separate concepts. With suspensions of suitably doped micelles or hydrophobic nanospheres we achieve short diffusion distances that are approaching those in homogeneous phase. Water insoluble recognition elements can now be used to effect sample concentration changes in analogy to homogenous reagents, and the combination of an ensemble of reagents may be used to develop functional toolboxes that are truly innovative. Examples will include the development of nanoscale probes for exhaustive ion sensing, the use of light to induce oscillating sample concentration changes in a selective and reproducible manner, the introduction of a nanoscale mimic of complexometric titration reagents with much improved selectivities, and potentiometric nanoscale sensing based on the incorporation of potential sensitive dyes.

Keywords: Analysis, Nanotechnology, Sensors, Titration
Application Code: Bioanalytical
Methodology Code: Chemical Methods
Nitric oxide (NO) controls many important physiological processes. Although NO’s role as a potent vasodilator is well known, NO is also very effective at preventing clotting and is a powerful antimicrobial agent produced by several innate immune cells (e.g., macrophages, neutrophils, etc.). Therefore, it should be possible to prevent infections and/or clotting widely associated with a variety of medical devices (e.g., intravascular catheters/sensors, urinary tract catheters, extracorporeal blood loops, etc.) by developing materials/devices capable of releasing low levels of NO gas over extended time periods. In this presentation, it will be shown that a variety of hydrophobic polymers (PVC, polyurethanes, silicone rubber, etc.) often utilized to fabricate biomedical devices/systems can be formulated/designed with relatively simple chemistries (including electrochemical approaches) to release NO gas for days and weeks with NO fluxes at or above those generated by many cells in our bodies. It will be further shown that such NO release materials/devices can be employed to make simple intravascular or urinary catheters that exhibit potent antithrombotic and antimicrobial activities when tested in vitro and in vivo (in animals). Finally, it will also be demonstrated that these new NO release materials/devices can be combined with more classical electrochemical analytical sensor technologies to create implantable sensors for oxygen, glucose, and lactate that exhibit greatly enhanced thromboresistance and thereby improved analytical accuracy when placed in veins or arteries for continuous monitoring of these important species in blood.
Detection of toxins is of particular importance because near real-time detection is essential in cases of imminent threat and because toxins can be modified or otherwise disguised while remaining active. The objective of this project is the creation of new, low-cost, appropriately sensitive paper diagnostic devices for the electrochemical detection of biological weapons. To satisfy this objective, we initiated development of a quantitative ricin sensor using a magnetic microbead supported silver nanoparticle (AgNP) metalloimmunoassay to probe the ricin A chain protein. The sensor integrates picomolar affinity antibodies with our easily handled, but sophisticated, electrochemical detection platform: the iSlip. The iSlip incorporates two simple, but effective, preconcentration steps. The first involves magnetic preconcentration of AgNP labels at a working electrode, followed by the spontaneous oxidation of these labels in the presence of a chemical oxidant stored on the device. Importantly, this oxidizing agent is delivered into the channel at a specific time and location by simply slipping a moveable piece of paper. The resulting Ag ions are then electrodeposited onto the working electrode (the second preconcentration step). Oxidation of this Ag layer leads to detection of the AgNP-labeled target. We have demonstrated quantitative electrochemical detection of ricin A chain at a LOD of 78 pM by means of fast (3.5 minutes) and non-enzymatic signal amplification. Importantly, the custom antibodies can bind to both glycosylated and recombinant ricin, allowing for the detection of genetically modified toxins. This project is sponsored by the Defense Threat Reduction Agency (Contract Number HDTRA-1-13-1-0031).
Non-invasive separation-free protocols are attractive for quantitative analysis in complex mixtures. To address the demanding selectivity issue, we propose to perform analysis under kinetic control upon exploiting the reactivity of labelling contrast agents. In particular, our simple analytical protocol has been applied in optical fluorescence microscopy, where autofluorescence, light scattering as well as spectral crowding presently bring limitations. We have introduced OPIOM (Out-of-phase imaging after optical modulation), which exploits the rich kinetic signature of a photoswitching fluorescent probe to achieve its selective and quantitative contrast. Filtering the specific contribution of the probe simply requires matching its photoswitching dynamics to the intensity and frequency of a modulated monochromatic light excitation. We validated OPIOM for selective imaging in mammalian cells and zebrafish, opening attractive perspectives for multiplexed observations under adverse conditions in biological samples.
Light and electrodes have a long history with spectroelectrochemistry, electrochemiluminescence and photovoltaics. Here we exploit light shined on an electrode to locally activate the electrode surface to allow Faradaic electrochemistry to occur on the illuminated spot only. The light is shined onto a modified silicon electrode where Faradaic electrochemistry is observed at defined locations whereas in the dark no electrochemistry is observed. To achieve this, first an oxide free silicon electrode is modified with a self-assembled monolayer of 1,8-nonadiyne which protects the surface against oxidation. Subsequently a redox species is attached to the surface, either a ferrocene derivative for n-type silicon or an anthraquinone derivative for p-type silicon. Provided at the potential at which oxidation/reduction of the redox species occurs the silicon is in the depletion, then no electrochemistry is observed in the dark. Upon illumination distinct Faradaic electrochemistry is observed. We show this electrochemistry can be confined to 50 µm with backside illumination and 30 µm with frontside illumination. Subsequently, using SECM we show that the surface bound redox species can be used as a mediator to detect redox species in solution. We next show that we can detect DNA hybridization and form DNA electrode arrays (an example of reading electrochemical information from the electrode surface) and show that we can write conducting polymers to the surface. Finally we demonstrate an application for the capture and localized release of cells from the surface.
Continuous synthesis of fine chemicals and pharmaceuticals using microfluidic platforms enables rapid investigation of reaction chemistry with small amounts of reagents and under conditions difficult to realize in batch. Current continuous flow systems are based on two basic approaches: 1) experimental setups consisting of plastic, glass or steel pipes that are easy to construct, replace and iterate, or 2) intricate setups made of silicon/glass which require the use of photolithography and micromachining, and taking significant resources to iterate. 3D printing of polymers, metals and glass can promise to combine the advantages of both types. Intricate setups can be designed and fabricated quickly and at a fraction of the cost compared to traditional machining methods. We describe the adaptation of an additive 3D printer to create reaction vessels for continuous flow chemistry. We explore the use of different materials to test their utility for additive printing, and their stability for handling the broad range of solvents and reagents used in flow chemistry. We use the thermoplastic, amorphous polymer acrylonitrile butadiene styrene (ABS) first to understand the capabilities of the 3D printer. The relationship between the extrusion temperature, bed temperature and extrusion rates are explored to make repeatable test objects. We then extend additive printing techniques to create polypropylene channels and reactors. We characterize the physical properties of the printed reactors. Finally, we study model chemical reactions of interest to the pharmaceutical community to study the dependence of reaction rates on channel geometry.
Oftentimes there is a gap between when a microfluidic device needs to be field tested in other labs and before it is ready for commercialization. The burden and high failure rate of fabricating PDMS based devices to disseminate to non microfluidic labs can hinder the adoption of these new devices. This is where 3D printing can help bridge the gap between proof of concept and commercial reality and allow new devices to be more rapidly disseminated and for microfluidics to more easily integrate into non microfluidic labs. Recently 3D printing has emerged as a method for directly printing complete microfluidic chips, although printing materials have been limited to non-oxygen permeable materials. We demonstrate the addition of gas permeable PDMS (Polydimethylsiloxane) membranes to 3D printed microfluidic devices as a means to enable oxygen control cell culture studies. Specifically, we use negative pressure to drive these devices with the integration of an additional 3D printed Venturi microfluidic device which allows us to pull desired oxygen conditions through the network using the oxygen condition as the compressed air source. The negative pressure has two main benefits; it reduces bubble formation in the culture chambers and serves to help hold the gas permeable membrane in place. We use this setup on three separate 3D printed microfluidic devices including a 100 mm petrie dish with integrated hose barbs, a 24 well plate insert, and a microfluidic dilution network able to generate 21 different stable oxygen conditions from two separate inputs.

Keywords: Air, Lab-on-a-Chip/Microfluidics
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
3D printing is a potentially attractive fabrication and prototyping method for microfluidic devices. In particular, there has been great interest in using commercially available 3D printers or 3D printing services to fabricate microfluidic devices. To date, however, only passive devices (i.e., no integrated valves or pumps) have been demonstrated. Moreover, material selection tends to be limited to commercially available resin formulations. In this presentation we report the fabrication and characterization of pneumatically-controlled on-chip valves in microfluidic devices with fluid channels having minimum dimensions approaching 100 [micro]m. We use an inexpensive projector-based stereolithographic 3D printer, the B9Creator v1.1 (B9Creations, LLC) with our own poly(ethylene glycol) diacrylate (PEGDA) resin formulation, which we have explicitly developed to exhibit low non-specific protein adsorption when polymerized. Initial testing of our non-elastomeric membrane valves indicates that the fluid pressure needed to open a closed valve is the same as the applied valve closure pressure. We are exploring a variety of valve dimensions and geometries to maximize valve robustness and longevity for potential applications in small volume bioassays.

Keywords: Biosensors, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Despite being an old technique, additive manufacturing has received significant hype because of the ability to create just about anything. Our research has focused on addressing the issue of how mass-consumer targeted printers can be used to create microfluidic devices. We have evaluated low-cost (<$5k) printers based on UV polymerisation using a digital micromirror device (DMD) projector and fused deposition modelling (FDM). Each approach has advantages and limitations for making flexible, 3D enhanced microfluidic devices. The use of these printers to create integrated microfluidic devices with advanced 3D analytical capability will be discussed.
Microfluidic devices have been a very useful and important tool in our group and others for studying cells and cellular processes. In particular, our laboratory has employed various devices for investigating blood components and interactions of these components with other cell types. Here, we will present recent results demonstrating the utility and efficiency of 3D-printed devices and how they are helping our group understand the chemistry and biology of stored red blood cells that are used in transfusion medicine. Specifically, this presentation will describe various fluidic devices that are used to examine modifications to solutions used to store red blood cells prior to transfusion. Current storage solutions are extremely high in glucose and are thought to impair cell performance upon transfusion. Our results, using a 3D-printed circulation mimic, show that solutions modified to have lower glucose levels are beneficial to the stored cells. In addition, we will show other devices that have been printed that enable us to investigate the deformability of the stored cells and how these cells may behave after transfusion back into a patient environment.

Keywords: Bioanalytical, Biological Samples, Biotechnology, Lab-on-a-Chip/Microfluidics
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Organs-on-chips (OoCs) bioreactors, which aim to replicate human cellular responses in a microfluidic environment, may lead to advances in systems biology and provide new avenues for drug development and testing. Microfluidic devices are needed to enable automated fluid handling to allow for increased OoC lifetime and online analysis of OoC health and behavior. This presentation will describe the use of commercial and fabricated pumps and valves to develop Perfusion Controller/ Microclinical Analyzers (PC/µCAs). One PC/µCA is a stand-alone perfusion controller with continuous on-line analysis and calibration assembled from microfluidic syringe pumps and valves from Labsmith, Inc. This instrument consists of numerous pumps and valves integrated with a microfluidic cell trap, a multi-analyte electrochemical array, and Synapt G2 and de-salter for on-line ion mobility mass spectrometry (IM-MS). This combination enables the multiplexing of microscopy, electrochemical monitoring, and on-line IM-MS to monitor cellular morphology and metabolic flux of small molecules and proteins over time into one platform. In a parallel study, fabricated rotary planar peristaltic micropumps (RPPM) and rotary planar valves (RPV) have been developed to perfuse OoCs for days to weeks within an incubator. As with the Labsmith-based PC/µCA, additional RPPMs and RPVs are utilized to sample fluid directly from the OoC and deliver this fluid, as well as calibration solutions, to an electrochemical sensor placed downstream. The RPPMs and RPVs are customizable, allowing for a range of flow rates in the pumps and input ports for the valves, and can be used in a variety of projects. The use of these pumps and valves in the development of a Neurovascular Unit on a Chip will be discussed. In summary, the automation, measurement capabilities, customizability, and abilities for long-term use make both the commercial and fabricated pumps and valves valuable tools for high-yield systems biology research.
Dielectrophoresis, the motion of a particle in a non-uniform electric field, is an emerging technology for identifying and isolating targeted mammalian cell populations. Here, we present a new technique, contactless dielectrophoresis (cDEP), which uses fluid electrodes isolated from the sample channel by a thin membrane to induce large electric field gradients. These fluid electrodes enable the creation of complex 3D electrode geometries and the production of high throughput sample channels.

cDEP systems are a complex network of resistive and capacitive elements which behave as a high-pass filter. Producing electric field gradients necessary for DEP within these devices is non-trivial and requires the use of high-voltage, high-frequency electrical signals and optimization of these devices is necessary if we wish to employ both positive and negative dielectrophoretic forces to separate cells. Multi-physics simulations were used to study the electro- and thermo-dynamics of these systems, predict particle trajectories, and trapping efficiencies prior to experimentation. These simulations have enabled us to develop systems which can manipulate mammalian cells between 1 kHz and 1 MHz in a completely sterile environment.

The cDEP platform has been used to study the electrical properties of numerous cell types. Interestingly, we found that the membrane capacitance of syngeneic ovarian cancer cells changed significantly as the cells progressed from benign to aggressively metastatic. Additionally, our team has shown the utility of cDEP to enrich and subsequently re-culture tumor initiating cell populations. The work presented will cover the conceptual optimization of cDEP devices, current experimental results, and future prospective.

Keywords: Biological Samples, Biomedical, Biosensors, Electrophoresis

Application Code: Biomedical

Methodology Code: Microfluidics/Lab-on-a-Chip
In this talk I will discuss two microfluidic systems that take advantage of the ability to control flow exquisitely. In the first set of applications, we designed a series of microfluidic devices that can systematically load and keep in place single suspension cells, embryos, and cell aggregates. These devices take advantage of the well-behaved hydrodynamics at the micro scale, and can manipulate particles without using moving parts and yet with high reliability. We demonstrate several applications using these systems for image-based cell assays. For the second set of applications, we designed devices for handling highly active biological samples – the genetic model system C. elegans – for high-throughput applications. Although these devices require active control, several design features largely reduce the complexity and enhance robustness of the operation of these devices. By computer automation and coupling with advanced image informatics, we demonstrated several applications using this system to perform high-throughput and high-content screens.

Keywords: Automation, Bioinformatics, Biomedical, Lab-on-a-Chip/Microfluidics

Application Code: Biomedical

Methodology Code: Microfluidics/Lab-on-a-Chip
Type 1 diabetes mellitus, an autoimmune disease resulting in destruction of the insulin-producing pancreatic beta cells, is one of the most common and costly chronic pediatric diseases. A significant impediment to understanding disease pathology and the development of cellular replacement therapies for Type 1 diabetes is the inability to sustain mature human beta cells in culture. We seek to engineer physiomimetic 3D niches within higher throughput microfluidics devices for maturation, maintenance and monitoring of human beta cells via the convergence of technologies from stem cell biology, matrix engineering, micro/nano fabrication, and microsensors. The microfluidic devices will connect to universal docks and provide intimate control over the cellular microenvironment by independent and simultaneous modulation of liquid and gas phases, multiparametric monitoring and assessment of cellular readouts and samplers for off-line biochemical analyses. With this degree of control, the effect of various niche parameters, such as the extra cellular matrix, nutritional gradients, soluble factors, and oxygen microenvironments on human islet maintenance and generation of mature islets from human pancreatic precursors can be clearly delineated. Of particular interest in this application are the contributions of the physiological and extracellular matrix environment on islet health and maturation. Physiological oxygen, a critical parameter in steering pancreatic progenitor differentiation towards endocrine lineage, can be intimately modulated on the microscale via the control afforded by the microfabricated platform. Further, systematic evaluation of the contributions of matrix components on promoting islet health and directing islet differentiation within controlled 3D niches is feasible via tailored presentation of native extracellular matrix components.
Microfluidics is a dynamic and rapidly growing field with potential applications in numerous areas, from food and water safety, to environmental monitoring and clinical analysis. Working on the microscale offers attractive advantages such as shorter processing times, low sample and reagent consumption and the possibility of portable systems.

Sample preparation is a challenging task, in particular for applications involving biological particles. Miniaturizing sample preparation opens the possibility for fully integrated systems. Important research efforts are being devoted towards the development of microscale techniques suitable for sample preparation. Dielectrophoresis (DEP), an electric field driven technique, offers great potential due to its flexibility and ease of integration. DEP has been successfully employed to significantly enrich, sort and separate samples containing DNA, proteins, cell organelles, virus and cells.

DEP is a transport mechanism driven by polarization effects when a dielectric particle is exposed to a spatially non-uniform electric field. DEP can be used with charged and neutral particles, employing AC or DC electric potentials. DEP offers more versatility than electrophoresis since it has the capability for significant particle enrichment.

This work is focused on insulator based DEP (iDEP), a dielectrophoretic mode that uses insulating structures between two external electrodes to create electric field gradients and generate dielectrophoretic forces on particles. In this presentation we will analyze the application of iDEP systems for sample preparation and demonstrate the potential of this novel technique for successful manipulation of biological particles, such as cells. This study includes experimental work and mathematical modeling with COMSOL Multiphysics. Experimentation was carried out with devices made from polydimethylsiloxane.
Advances in Separation with Capillary Liquid Chromatography

Resolving DNA Using Narrow Bare Open Capillaries

In this presentation, I will introduce an innovative approach to resolve DNA using a narrow bare open capillary. When DNA are transported inside a narrow open capillary under a pressure-driven condition, DNA molecules move as particles. Larger DNA fragments have greater effective diameters and cannot go to the capillary wall (the slow-moving region) as close as smaller fragments can and, therefore, they move faster. On the basis of this principle, DNA fragments of a wide size range can be separated with resolutions comparable to gel electrophoresis. Because this technique uses Bare Narrow Capillaries and its separation mechanism is primarily HydroDynamic Chromatography, we call it BaNC-HDC. I will start by telling briefly how this technique was evolved, I will then show the major accomplishment we have achieved, and I will also share with the audience our thoughts on the future of this technology.

**Keywords:** Bioanalytical, Biotechnology, Capillary LC, Separation Sciences

**Application Code:** Genomics, Proteomics and Other 'Omic

**Methodology Code:** Other
Chromatographic separation of proteins is a formidable, long-term challenge because even one protein is a complex mixture due to post-translational modification. Consequently, a sample having just one protein that was purified by antibody capture can have hundreds of proteoforms. Multidimensional separations have been used to address this challenge in proteomics and also in quality assurance of protein drugs. In our work, we focus in increasing the resolution in each constituent separation. We have particularly given attention to using submicrometer particles in chromatography to reduce broadening arising from mass transport and packing heterogeneity. We discuss how far this technology can be pushed for real samples and commercially available instruments.

Keywords: Bioanalytical, HPLC Columns, Peptides
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
The present contribution aims at illustrating and demonstrating how micro-machining technology can give a boost to Liquid Chromatography (LC). Currently, LC is routinely used in nearly every chemical analysis lab. Despite its high degree of maturity, the technique however suffers from serious performance limitations when faced to the complex samples that need to be separated to solve the current state-of-the-art problems in the biological and pharmaceutical research (e.g., proteomics and metabolomics), the food and environmental analysis, etc.....

Currently, LC is conducted in packed particle columns. As this packing inevitably is disordered, the flow pattern in these columns is to a large extent disordered as well, in turn leading to an unnecessary loss in separation efficiency. To solve this packing disorder problem, the present contribution focuses on the possibilities of advanced photolithographic etching techniques such as the Bosch-process to produce perfectly ordered porous support columns with optimized hydrodynamic shape and optimized external porosity.

The work presented here in fact aims at putting to work an original idea of Fred Regnier wherein he advocated the use of micromachining to produce perfectly ordered chromatographic supports. Since then, the technological possibilities have clearly evolved and it is now possible to fabricate pillar array columns with a very tight (i.e., sub-micron) control of the pillar to pillar distances and the pillar dimensions.

At the conference, we will demonstrate the possibility of rapid multi-component separations and the possibility to achieve very low plate heights with a microfabricated column in pressure-driven LC.

The possibility to combine the pillar array technology with the recipes used to produce monolithic columns, leading up to a brand new class of column technologies, will be highlighted as well.

Keywords: Chromatography, Liquid Chromatography
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Present day commercial UHPLC columns are typically 5 to 15 cm long, packed with sub-2 micron particles, and operated with upper pressure limits of 1,000 to 1,500 bar (15,000 to 22,000 psi). We have developed a system for capillary UHPLC columns with operating pressures to 3,400 bar (50,000 psi) and with elevated temperatures. This opens up the possibility for developing capillary columns with still smaller particles (down to 1 micron) and/or longer columns packed with sub-2 micron particles. We are routinely packing columns 25 cm long packed with 1 micron particles yielding 125,000 plates, 100 cm long packed with 1.5 micron particles yielding 350,000 plates, and 200 cm long packed with 1.9 micron particles yielding 600,000 plates. The shorter columns with 1 micron particles offer very significant performance advantages over existing HPLC and UHPLC columns but with UHPLC type speeds. The longest columns packed with 1.9 micron particles require analysis times of hours, but provide extremely high separation efficiencies and peak capacities in return. All of these columns are well suited for separations of peptides in combined LC-MS-MS proteomics applications. The range of particle sizes and column lengths provides a wide spectrum of speed vs. separation power permitting us to match the separation column to the difficulty of the particular separation problem at hand.

Keywords: Bioanalytical, Capillary LC, Liquid Chromatography/Mass Spectroscopy, Peptides
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Guidelines for high-speed chromatography are by now well understood for the most widely used columns (e.g., 2.1 mm ID). When sample size is limited and concentrations are near the detector’s detection limit, then capillary columns must be used to avoid sample dilution and even achieve sample concentration on-column. Under these circumstances, it is necessary to optimize the column diameter as well as particle diameter and column length given a certain pressure and theoretical plate requirement (N). The goal is to squeeze a sample with a volume $V_s$ into a peak with a volume $V_p$ where $V_p/V_s$ is as small as possible without suffering from volume overload which degrades N. With this approach, we have found conditions for making online microdialysis measurements for dopamine or serotonin over many hours with one-minute time resolution. By using superficially porous particles and temperature assisted solvent focusing, we are able to improve the method significantly.
This talk will discuss a multi-dimensional multi-channel micro-gas chromatography ([micro]GC) system and one of its important components, non-destructive flow-through gas detector, which makes the multi-dimensional [micro]GC possible. We will first present the multi-dimensional [micro]GC design and describe its performance. Then we will introduce various GC detectors developed in our lab in the past few years, including graphene nanoelectronic detectors and metallic nanoparticle detectors, as well as optical ring resonator detectors and photo-ionization detectors. Advantages of those detectors in comparison with other existing detectors will be emphasized. Finally, we will show examples of the potential applications of the multi-dimensional [micro]GC, if time permits.

Keywords: GC, GC Detectors, Sensors
Application Code: Bioanalytical
Methodology Code: Gas Chromatography
Metal-Organic Frameworks (MOFs) are a new class of crystalline materials exhibiting tailorable micro-to-mesoporosity. Comprised of metal ions linked to organic electronic donors such as aromatic amines and carboxylates, these materials possess a high degree of synthetic versatility that enables them to be used as recognition materials when integrated with a sensing platform. Sensing concepts using MOFs that adsorb small molecules to generate changes in mass, strain, electrical resistance, and luminosity have been demonstrated, for example. Here, we describe three novel MOF sensing modes that we are developing: First, we will discuss the use of MOFs for detecting molecular oxygen. Although O2 detection by quenching MOF luminescence is feasible, this approach has low sensitivity relative to mass-based sensors such as surface acoustic wave (SAW) devices. We describe a process for growing oxygen-sensitive MOF thin films and demonstrate O2 detection using a SAW sensor. Second, a less explored sensing approach using MOFs is colorimetric detection. We describe a colorimetric small-molecule sensor concept based on a composite comprised of a MOF powder embedded in an organic polymer. In this architecture, a MOF containing exchangeable metal coordination sites that bind small molecules is used as the sensing element. Upon binding, the electronic energy levels of the MOF are perturbed, leading to an easily detectable change in color. We demonstrated this concept using a MOF known as HKUST-1, which changes color upon adsorbing molecules that coordinate to the copper dimers in its structure. Finally, present a new sensing concept, in which analyte adsorption induces multiple response modes within a single MOF. The concept is demonstrated using an electrically conducting MOF film grown on MEMS devices, enabling transduction by measuring both mass uptake and the change in electrical conductivity.

Keywords: Adsorption, Chemical, Materials Science, Nanotechnology
Application Code: Other
Methodology Code: Sensors
MOFs are a class of inorganic-organic hybrid materials with high porosity and massive internal surface area. The tunability of organic linkers and metal cluster SBUs make MOFs of great candidates for variety of applications. Most of the reported fluorescent MOFs are not suitable for sensing purposes due to their low chemical stability. Zr-MOFs have been acknowledged for their high stability, which possess great potential for chemical sensing purposes.

In the course of targeting stable MOFs, we have constructed a series of porphyrin containing Zr-MOFs, PCN-22X, with exceptional stability and applications. Most of the PCN-22X MOFs were found to retain their frameworks after emerging into aqueous solutions with pH ranging from 1 to 11. As a remarkable example, PCN-222 was proved to remain intact after the treatment of concentrated HCl. The pH dependent fluorescence of PCN-225 was investigated and was concluded that the fluorescent intensity of PCN-225 is largely influenced by the protonation and deprotonation of the nitrogen atoms in porphyrin centers. The results indicated that PCN-225 is a promising material for pH sensing, especially in the pH range of 7–10.

We have recently reported PCN-94, which constructed from Zr6 clusters and H4ETTC linkers, with ftw topology. This MOF showed extraordinary fluorescent properties with a 99.9% ± 0.5% quantum yield under argon. By using the same linker, PCN-128, which possesses a csq topology, was also constructed. A novel piezofluorochromic behavior of PCN-128 was discovered, in which PCN-128 undergoes a phase transition under chemical or physical stimulations. This phenomenon was observed from the color change as well as the change in emission spectra. Interestingly, the transformation of the material can be monitored stepwise by fluorospectroscopy. Both PCN-94 and PCN-128 show very high stability and high fluorescent sensitivity against their environments which makes these material of great candidates for chemical sensing uses.
**Session Title**: Gas-Phase Bioanalytical Diagnostics Using Sensing Nanomaterials

**Abstract Title**: Artificially Intelligent Nanoarrays for Non-Invasive Detection of Disease

**Primary Author**: Gady Konvalina

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**Abstract Text**

An emerging approach for diagnosing diseases relies on profiling volatile biomarkers that are emitted from cells in the affected area. These volatile biomarkers can be detected either via the exhaled breath or from the skin, without going invasively into the human body. The current talk will present solid-state and flexible arrays of artificially intelligent nanomaterial-based sensors that are trained in their ensemble to detect such volatile disease biomarkers. The development and the use of these systems to identify high-risk groups for specific diseases and genetic mutations and to monitor the therapy provided to people affected by that disease will be presented and discussed.

**Keywords**: Array Detectors, Biomedical, Lab-on-a-Chip/Microfluidics, Sensors

**Application Code**: Biomedical

**Methodology Code**: Sensors
Vapor sensors have their niche in gas-phase bioanalytical diagnostics when an unobtrusive, low-power, and cost-sensitive technical solution is required. Unfortunately, existing vapor sensors often degrade their vapor-quantitation accuracy in the presence of high levels of interferences and cannot quantitate several components in complex gas mixtures. Thus, new sensing approaches with improved sensor selectivity are required. Our presentation will demonstrate that this technological task can be accomplished by the careful design of sensing materials with new performance properties and by coupling these materials with the suitable physical transducers that have multivariate response. We will demonstrate the capabilities of our developed multivariable sensors for selective vapor sensing. We have developed diverse transducers based on electrical, mechanical, and optical readout principles that provide vapor-response selectivity previously unattainable by using other sensing approaches. This ability for selective vapor sensing provides opportunities to significantly impact the major directions in development and application scenarios of vapor sensors.
A suite of mass-spectrometry-based approaches are described that allow the investigation of individual neurons and small brain regions for their metabolites and peptides. Direct single cell MALDI-MS, SIMS-MSI, and MALDI-MSI provide unmatched information on the contents of the cells in the brain. Single cell MALDI-MS has been used for the discovery of hundreds of novel peptides and in the characterization of the heterogeneity of cellular populations. While this approach typically requires manual sample preparation and therefore is low throughput, it provides unmatched performance. MSI has evolved into a sensitive approach capable of probing tissues at cellular-level resolutions. We have adapted MSI to dissociated cell populations and hyphenate it to other information rich measurement capabilities. MALDI-MSI can acquire thousands of mass spectra from cell-sized samples, and can even be used to directly acquire spectra only from cells and not the spaces between them.

The high sensitivity of MALDI-MS, requiring attomole to zeptomole amounts of analyte, allows a range of analytes to be characterized in individual cells. Several applications of single cell MSI are highlighted including metabolite profiling of selected cells. Using these approaches, literally hundreds of new neuropeptides have been characterized in well-defined neuronal networks, and in several cases, the functional roles of these molecules described. MSI and dynamic sampling of the extracellular environment are used for elucidating novel cell to cell signaling molecules in a range of neuronal model systems. Current efforts involve extending the depth of chemical coverage and adapting these analytical approaches to higher throughput single cell assays. Our overarching goal is to uncover the complex chemical mosaic of the brain and pinpoint key cellular players in physiological and pathological processes.

Keywords: Mass Spectrometry, Method Development, Peptides
Application Code: Neurochemistry
Methodology Code: Mass Spectrometry
Imaging Mass Spectrometry of Biological Samples

A New Dimension: Ambient Mass Spectrometry of 3-Dimensional Objects by a Vision System-Directed Robotic Sampling Probe

In order to image biological tissues by mass spectrometry, samples typically must be sliced into a series of 2D sections to accommodate ion source geometry restrictions. During this process, there is potential for disturbing the natural spatial distributions of molecules of interest. Instead of altering the sample to fit the method, a new technique for imaging irregular, 3D surfaces was developed using a robotic sample introduction/ionization system for mass spectrometry termed Robotic Plasma Probe Ionization Mass Spectrometry (RoPPI-MS). In RoPPI, a Universal Robots UR5 robotic arm with six degrees of freedom maneuvers an acupuncture needle to probe the sample surface, then places the probe into the ionization region of a plasma ion source for subsequent MS detection. The motion of the robot is guided by a PrimeSense Carmine short-range sensor communicating to an RViz software package that generates a point cloud within for x,y,z coordinate selection on the sample surface. By probing multiple points across a sample, a chemical image of the surface may then be rendered. Direct analysis in real time (DART) was selected as a plasma-based ionization source for RoPPI given its ability to ionize both polar and non-polar analytes and its relatively large ionization region. The modularity of the RoPPI system makes it conducive to the imaging of various samples such as irregular mineral surfaces, biopsies, whole organs, or skin lesions, through any number of interchangeable sampling/ionization probes.

Keywords: Imaging, Mass Spectrometry, Sample Handling/Automation, Surface Analysis
Application Code: Other
Methodology Code: Mass Spectrometry
As lipids are the most common biomolecules in the brain after water, we studied brain lipid profiles using mass spectrometric imaging, to follow the molecular progression of Traumatic Brain injury in a mouse model of Blast Induced Traumatic Brain Injury (bTBI) and a rat model of controlled cortical Impact (CCI), in order to find biomarkers for early detection and treatment and the biochemical pathways involved.

We developed a new MALDI matrix that allowed us to detect all gangliosides, which only make up 6% of all lipids in the brain. The ganglioside GM2, which is present in the brain at a concentration of 0.08% increased after blast and REMAINED elevated. A surprising finding, as GM2 is only expressed in embryos, between weeks 17 and 21, in cortical and other neurons, during dendritogenesis. The only pathological condition where it is detected is a gangliosidosis due to mutations that lead to hexosaminidase A deficiency. The absence of this hydrolytic enzyme, whose alpha subunit is able to hydrolyze GM2 to GM3 due to a key residue, Arg424 which is essential for binding the N-acetyl-neuraminic acid residue of GM2. [Woods AS et al. Gangliosides and Ceramides Change in a Mouse Model of Blast Induced Traumatic Brain Injury. ACS Neuroscience 4, 594-600 (2013).]

In the case of CCI we developed a new technique, where we implanted tissue with silver, and found that in this type of trauma, it’s the sphingomyelins that increased, indicating that a different pathway is involved. We are in the process of studying the lipid profiles of the CSF of these animals, to establish a correlation between body fluids and brain tissue profiles. In addition nanoparticulate implantation has allowed us to get perfect reproducibility. In addition, we are testing a peptide drug, which has given promising results.

**Keywords:** Bioanalytical, Biological Samples

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
Direct measurement of metabolites from diseased tissue remains a challenge owing to structural diversity of the large number of primary and secondary metabolites present. These metabolites represent the climax of all physiological responses and reflect ecological relationships between pathogenic microbes and human response. Strategies aiming at direct measurement of metabolites will enhance our understanding of the roles that these molecules play and may result in emergence of new molecules and novel biosynthetic pathways. Untargeted metabolomics employed for such studies generates large volume of datasets that are rich in information hampering comprehensive analysis. Recent advancements in mass spectrometry and bioinformatics tools have reinvigorated the field of untargeted metabolomics of complex samples. Herein, we employ high-scan-speed QTOF coupled with ultra high performance liquid chromatography for data collection combined with molecular networking as organizational tool, cytoscape and spatial mapping as visualization tool, and automated database search for rapid identification of metabolites.
Examining Pharmacokinetics and Pharmacodynamics in 3D Cell Cultures by Imaging Mass Spectrometry

Three dimensional cell cultures are attractive models for biological research. They combine the flexibility of cell culture with some of the spatial and molecular complexity of tissue. For example, colon cancer cell lines form spheroids, in vitro mimics of poorly vascularized tumors. The spheroids are composed of a central necrotic core, a middle quiescent layer and an outer proliferative layer of cells, similar to a rapidly growing colon tumor. Our laboratory has characterized the distribution of endogenous proteins via MALDI imaging mass spectrometry in colon spheroids and determined that the molecular gradients correlate with the pathophysiological changes in the structure. We have also developed an approach to evaluate the penetration of compounds into 3D cell cultures. Most novel drugs are initially evaluated with 2D cultures before moving directly to costly animal studies. 3D cultures, like spheroids, provide an ideal testbed to minimize these studies. Working with the chemotherapeutics oxaliplatin and irinotecan, our data supports differential penetration of these clinically relevant drugs into spheroids. We can either perform traditional imaging mass spectrometry or add a complementary analysis of enzymatically generated concentric rings of cells via serial trypsinization. In this procedure, radial layers of cells are sequentially removed, analogous to “peeling an onion”, and then analyzed via nLC-MS/MS. We are now evaluating the pharmacokinetics and pharmacodynamics of the spheroids with clinical treatments. We dynamically treat spheroids in 3D printed microfluidic devices and then use imaging mass spectrometry to explore the molecular distributions of the prodrug and its metabolites. Our future studies include evaluation of drug libraries to evaluate the functional moieties that contribute to the penetration of compounds, including the development of novel statistical workflows to evaluate imaging data generated from 3D cell cultures.

Keywords: Bioanalytical, Biopharmaceutical, Imaging, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Target-specific drug delivery that treats cancers but leaves normal tissue unharmed, is the ultimate goal of cancer therapy. Nano-sized drugs have virtually limitless synthetic possibilities enabling a variety of payloads to be delivered to the tumor resulting in effective therapy. Tumors have relatively higher concentrations of nano-sized drugs than normal tissue due to the leaky nature of tumor vasculature, a phenomenon known as enhanced permeability and retention (EPR). The EPR effect, while permitting an increase in intratumoral nano-drug concentration, nonetheless has a limited ability to achieve concentrations that take full advantage of the capabilities of nano-sized drugs. Thus, a method for better nano-drug delivery might lead to improved cancer therapy. We have recently developed a new type of highly selective, molecularly-targeted cancer therapy, named photoimmunotherapy (PIT), that is based on conjugating a near infrared silica-phthalocyanine dye, IR700, to a monoclonal antibody (MAb) thereby targeting cancer-specific cell-surface molecules. After the administration of the conjugate and the targeted application of light, the intratumoral vascular barrier is significantly disrupted enabling a dramatic (up to 24 fold) increase in nano-drug concentration in PIT treated cancer tissue compared with non-treated control tumors. In this lecture I will discuss general pharmacokinetic characteristics of nano-sized molecules in the body, especially focusing on drug delivery in cancer tissue and routes of excretion that are important for improving the safety profile. In addition, I will discuss the basis and applications of the PIT-induced super-enhanced permeability and retention (SUPR) effect that could dramatically improve nano-drug delivery thereby enhancing the therapeutic effects of nano-sized anti-cancer agents.

Keywords: Biomedical, Biotechnology, Nanotechnology, Near Infrared
Application Code: Biomedical
Methodology Code: Near Infrared
Genomic information flows from DNA to RNA and from RNA to peptides and proteins; therefore, genomic information is stored in both the base sequences of DNA and RNA and in the amino acid sequences of peptides and proteins. Chemically modified bases and amino acids are particularly important in the expression of life functions and disease markers. However, no existing sequencing technologies can directly sequence RNA and peptides, or detect chemically modified molecules. Consequently, innovative methods for sequencing DNA, RNA, and peptides as well as detecting their chemical modification will greatly advance the fields of molecular biology, medical science, and drug development. In this lecture, I will report a single-molecule sequencing method based on single-molecule conductance measurements. Single-molecule conductance can be determined by tunneling currents created when single bases and amino acids pass through nanogaps. This method sequences DNA, RNA, and the partial amino acid sequences of peptides by detecting the differences in single-molecule conductances of the constituent molecules. In addition, this method detects chemically modified bases and amino acids at single-molecule resolutions. Because nanodevices for single-molecule sequencing technologies are fabricated using semiconducting technologies, the sequencing technologies are expected to realize label-free, ultra-low cost, and high-throughput genomic information sequencers for use in personalized medicine. Toward this goal, we have launched a venture company, Quantum Biosystems. Our company is currently working on the development of single-molecule DNA sequencers.
Identification and quantification of substances in biological samples is a subject of great interest today, particularly in drug metabolism and pharmacokinetics, biomarker discovery and validation, and clinical diagnostics. The fact that mass spectrometry (MS) can analyze hundreds of metabolites, drugs, and proteins in milliseconds suggest it could vastly accelerate decision making. But mass analysis is preceded by a lengthy set of sample preparation steps involving sampling, the removal of cells or cellular debris, taking a sample aliquot, chemical modification in some cases, analyte enrichment, and selection of a portion of the sample for MS analysis or archiving. Collectively this requires hours and involves multiple sample transfers. Clearly, sample preparation limits the potential of MS.

Keywords: Bioanalytical, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Other
LC-MS/MS has rightfully earned a prominent position in pharmaceutical, toxicology, and clinical analysis due to its enhanced sensitivity and increased dimensions of analysis. The quantitative analysis of multiple protein biomarkers is an emerging area in biological mass spectrometry research. The translation of discovery-based data into targeted quantitative analysis is critical for the successful advancement of biomarker research projects. Typical workflows require either many methods for optimization or select only a small number of peptides for quantitative analysis. A procedure for rapid method optimization is described, with a goal to achieve specific, reproducible, and efficient multivariate quantitative analysis.

Keywords: Biological Samples, Biopharmaceutical, Clinical Chemistry, Liquid Chromatography/Mass Spectrometry

Application Code: High-Throughput Chemical Analysis

Methodology Code: Liquid Chromatography/Mass Spectrometry
Session Title: JAIMA - The State-of-the-Art Technologies from Japan: Analytical Instruments with/for Nano-Bio Techn
Abstract Title: Biological Applications of Fine Structure Analysis and Peripheral Technology Using Cryo-Scanning Electron Microscopy

Primary Author: Yuji Hasebe, JEOL Ltd.

Co-Authors: Kazuteru Kawauchi, Toshiaki Suzuki, Yoshiko Takashima

Date: Tuesday, March 10, 2015 - Afternoon
Time: 04:10 PM
Room: 260

Abstract Text:
A Cryo-SEM can be used for observation of specimens containing water by freezing. The Cryo-SEM used in this experiment is composed of a Cryo preparation chamber and a cooling stage. Recently, the spatial resolution at low voltages of an SEM, which can be used with a Cryo-SEM has been improved. You can observe specimens in a low vacuum mode with a Cryo-SEM. We used the Quorum Technologies Cryo unit PP3000T on the JEOL FE SEM JSM-7100F as a Cryo-SEM. Observation results of biological and food specimens containing water are reported.

Keywords: Analysis, Biological Samples, Microscopy
Application Code: Biomedical
Methodology Code: Microscopy
Optical Probes of Living Systems with Single Cell and Single Molecule Resolution

Live-Cell Super-Resolution Microscopy at <100 nm Resolution: Development and Application

Nanoscopy (super-resolution) techniques such as STED and FPALM/PALM/STORM microscopy utilize either targeted or stochastic switching of fluorescent molecules to achieve ~25 nm spatial resolution – about 10-fold below the diffraction limit. However, their primary application has been focused on fixed samples because of a lack of suitable live-cell compatible labels and time resolutions that have been limited especially for FPALM/PALM/STORM to seconds to minutes [1].

In this talk, I will present recent advances in live-cell nanoscopy. Newly developed probes, labeling procedures and instrumentation optimized for live-cell imaging have expanded the application range of live-cell STED microscopy. Using recently introduced sCMOS cameras in combination with a set of novel algorithms, we have shown acquisition speeds of up to 32 super-resolution images per second are possible in FPALM/PALM/STORM [2].

I will present the underlying technologies and demonstrate their performance with cell biological applications demonstrating the versatility of live-cell nanoscopy.

Disclaimer: J.B. declares financial interest in Bruker Corporation.


Keywords: Biomedical, Fluorescence, Instrumentation, Microscopy

Application Code: Biomedical
Methodology Code: Microscopy
A conventional fluorescence microscope is limited in the range of spatial frequencies, and therefore detail, that it can transmit. This limit is defined by the numerical aperture of the microscope’s objective lens and wavelength of light used for imaging. Structured illumination microscopy (SIM) expands the frequency response of the system by exposing the sample to patterned excitation illumination. The illumination pattern combines with sample structures to create Moiré fringes, optical artifacts representing previously unobservable frequencies. By patterning the illumination laterally and axially, a two-fold improvement in x, y, and z dimensions is achieved. Current implementations of SIM are capable of fast illumination pattern generation and data acquisition rates. Also, SIM requires relatively low illumination intensities compared to most other super resolution technologies and works with commonly used dyes and fluorescent proteins. Therefore, this method is well suited for both fixed and live cell super resolution imaging applications.

Keywords: Biological Samples, Biomedical, Fluorescence, Microscopy
Application Code: Biomedical
Methodology Code: Microscopy
By definition, living specimens are animate. Therefore, a full understanding of dynamic biological systems will only be obtained by observing them with enough 4D spatio-temporal resolution and for a sufficient duration, to capture the phenomena of interest. Unfortunately, conventional widefield or confocal microscopes are either too slow, lack the spatial resolution, or induce too much photodamage to meet these requirements. To address these limitations, we have developed a new approach for sub-cellular light-sheet microscopy capable of imaging fast three-dimensional (3D) dynamic processes in vivo at signal to noise levels typically obtained only in total internal reflection fluorescence (TIRF) illumination. By utilizing 2D optical lattices, we generate a thin (~400 nm full width half maximum) plane of light coincident with the focus of a high NA detection objective. Using this technique, we demonstrate substantial advantages in speed, sensitivity and reduced phototoxicity compared to conventional point scanning and spinning disc confocal microscopes as well as light-sheet microscopes utilizing single Gaussian or Bessel beams. We leverage these advantages to image samples ranging over three orders of magnitude in length scale from single molecules to whole embryos. Specific examples to be presented include: 3D single molecule tracking of fluorescently tagged transcription factors in densely labeled embryonic stem cell spheroids, 3D imaging of microtubule growth phases and organelle dynamics throughout the course of cell division, rapid 3D imaging of Tetrahymena motility at over 3 volumes per second, and 3D protein localization throughout the course of dorsal closure in Drosophila embryos. By combining lattice light sheet microscopy with novel fluorescent probes, we will also demonstrate 3D super-resolution localization microscopy with unprecedentedly rich detail over large fields of view and in thick 3D samples such as dividing cells and small embryos.
Over the past decade several new far-field super-resolution microscopy methods have emerged which overcome the diffraction limit, improving the spatial resolution to the nanometer scale. Stimulated emission depletion microscopy achieves super-resolution purely optical by engineering the point-spread function through a second red-shifted, donut shaped depletion beam. STED microscopy provides easy and intuitive access to multi-colour super-resolution as well as live cell imaging and has successfully been applied and gave novel insight in cell biology and neurobiology.

This presentation explains the basic principle of STED, summarizes the development of STED microscopy and illustrates the different STED modalities - STED CW, pulsed STED and gated STED. Application examples of 2D- and 3D-STED with fluorescent proteins as well as standard organic fluorophores will be shown. Furthermore I present recent developments in labelling strategies and discuss their potential for live-cell STED imaging.

Keywords: Fluorescence, Imaging, Microscopy
Application Code: Other
Methodology Code: Microscopy
In the last two decades the evolution of the scientific method has moved traditional disciplines forward and has led to the development of multiple completely new fields. In each case the unifying change has been the continued expansive integration of technologies on all fronts, including molecular, biochemical and computer based. Few fields of endeavor have embraced these changes as much as microscopy. At all levels in the last 20 years there has a massive and continuous expansion of the capabilities of the microscope on all fronts. The current research microscope represents the integration of modern optics, robotics, computing, probes and cameras. The impact of modern imaging on our understanding of disease and the potential for therapeutics has been has been extreme, particularly as we continue to expand scientific progress towards discovery rather than reductionist approaches. Equivalently there has been an explosion in the probes suitable for use in the modern microscope such that single molecules can be chased in 3 dimensional space and the local environment assessed in real time. Integrating novel fluorogen based probes into highly sensitive and extremely fast high through put screens to define protein, delivery and function in 3D space at high speed and with absolute certainty of localization has been the focus of our work. This seminar will discuss these technologies and their development as we attempt to develop methods to specifically develop assays to unravel molecular fates within cystic fibrosis and the specific functional sites of therapeutic correctors within living cells.

Abstract Text

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Keywords: Microscopy
Application Code: Biomedical
Methodology Code: Microscopy
Eurofins CAL performs contaminants and residue testing for the import/export market, providing services to leading global feed, food and beverage retailers and producers. The methods offered include pesticide residues, industrial contaminants, multi-residue mycotoxins, medicated feeds testing and antibiotic residues. In this environment of rapidly changing customer needs, we must fully utilize the technical capabilities of the instrumentation to increase application scopes of our methods without the luxury of time for method development. To meet our clients needs we are continually adding new analytes to existing screens and applying existing methods to a wide-variety of matrices. LC-MS/MS and GC-MS/MS applications are essential to high-volume, fast throughput testing and instrument capacity (run time) also is a key factor. Sample preparation and clean-up are frequently the rate-limiting steps in terms of production. Examples of current challenging matrix / analyte combintions and approaches for resolution will be discussed.

Keywords: High Throughput Chemical Analysis, Quantitative, Sample Handling/Automation, Solid Phase Extracti
Application Code: Food Contaminants
Methodology Code: Sampling and Sample Preparation
# Current Topics in Analytical Food Analysis

## How Can it be Hard to Make a QuEChERS Kit?

It has been seven years since the widespread acceptance of the AOAC and EN versions of the “QuEChERS” sample preparation protocol for food analysis and over that time about a dozen companies have entered the market with consumable kits to address these specific methods. These kits seem frightening simplistic with salts in packets and common centrifuge tubes such that one would think it is easy to manufacture and sell these kits. It is just salt in a tube, right?

This presentation discusses some of the unexpected and unknown problems that can arise in setting up to manufacture a QuEChERS product. Issues with product quality related to sourcing and process with discussed along with solutions learned along the way. This somewhat entertaining path also has let to useful insights on the methodology itself and how one can potentially improve their use of sample preparation techniques for LC/MS and GC/MS analysis.

**Keywords:** Food Contaminants, Food Safety, Sample Preparation, Separation Sciences

**Application Code:** Food Contaminants

**Methodology Code:** Sampling and Sample Preparation
Strategies used to develop LC-MS/MS based methods for vitamin analysis in food and dietary supplements will be presented by discussing some of the relevant case studies. First case study is a LC-MS/MS based method development and validation of the analysis of different folate vitamers. Schemes and strategies used in the method development and optimization of the relevant parameters including chromatographic conditions, mass spec parameters and extraction and solid-phase extraction conditions will be discussed. The strategies employed in evaluation of the method precision and accuracy will also be presented. The second case study will similarly discuss studies employed to develop and validate a LC-MS/MS method of analysis of vitamins D2, D3, 25(OH)D2 and 25(OH)D3 in various food and pet food matrices employing an extraction method more efficient than the conventional extraction methods.
With food regulations continuing to expand, labs are required to monitor more compounds in more commodities, and with consumer drivers to survey for other non-targeted potential hazards that could arise, there is an increasing demand for analytical techniques and methods that enable identification and quantitation of targeted, regulated compounds with capabilities for non-targeted surveillance of other unknowns. The advent of MS/MS libraries and high resolution / accurate mass instruments are now capable of performing both targeted and non-targeted screening in a single LC-MS/MS run. In this presentation, we will show the development of a workflow using a very generic extraction procedure and full scan MS/MS and high resolution MS and MS/MS data acquisition to confidently identify and accurately quantify targeted chemicals based on retention time, accurate mass, isotope pattern and MS/MS library searching. In addition, the implementation of sample-control-comparison was used to enable routine labs to successfully survey samples for unexpected contaminants in addition to targeted lists of known regulated compounds. Identification was based on high resolution MS and MS/MS information, including empirical formula finding, ChemSpider searching, and automatic MS/MS fragment ion interpretation. The automation of this challenging data processing workflow allows easy result review and reporting for practical implementation of either QTRAP or high resolution QTOF mass spec workflows for routine food contaminant screening in the modern food laboratory.

Keywords: Beverage, Food Contaminants, Food Safety, Liquid Chromatography/Mass Spectroscopy
Application Code: Food Contaminants
Methodology Code: Liquid Chromatography/Mass Spectrometry
### Current Trends in Pharmaceutical Dissolution Testing

**Applications of USP Apparatus 3: The Reciprocating Cylinder**

The development of the Reciprocating Cylinder Apparatus arose from a need for an alternative drug release apparatus capable of providing pharmacokinetic and mechanical conditions that more closely represented the various regions throughout the gastro-intestinal tract. While the traditional paddle and basket apparatus offered a convenient means to evaluate most oral drug formulations at single and multiple pH and over long periods it was difficult to change pH during the test and changes in agitation rates during the in-vitro test are seldom noted. The material presented in this session not only shows the compliance of the apparatus to meet more biorelevant requirements above but, illustrate the versatility of the apparatus to test products from immediate release to extended release as well as poorly soluble compounds, chewable formulation and numerous modified release products. Several case studies will be presented.

**Keywords:** Dissolution, Drugs, Instrumentation, Pharmaceutical

**Application Code:** Pharmaceutical

**Methodology Code:** Other
Current Trends in Pharmaceutical Dissolution Testing

Biphasic Dissolution

A review of biphasic dissolution instrumentation and results, including a comparison of various instrumentation configurations. The application of biphasic dissolution in general will also be discussed relative to the drugs and formulations where biphasic dissolution may be beneficial.

Keywords: Dissolution, Drugs, Quality Control
Application Code: Pharmaceutical
Methodology Code: Other
The ability to monitor the concentration of a solution in real time with in situ monitoring instrumentation has significant advantages over traditional techniques which require the sample to be removed for analysis. This is especially true when measuring a kinetic environment like a dissolution test. Fiber optic UV probes have been used for this analysis for close to 15 years and are a widely accepted technique in the drug development tool box. As drug product makes its way through the development process, the need for this technique to be used in a validated environment becomes necessary. Controlling variables and other special considerations need to be taken when validating an in situ technique type of analysis whether UV or any other technique used to measure concentration within the vessel.

**Keywords:** Dissolution, Fiber Optics, Ion Selective Electrodes, Validation

**Application Code:** Pharmaceutical

**Methodology Code:** UV/VIS
Current Trends in Pharmaceutical Dissolution Testing

Dissolution Testing for n=12

New drug applications (NDAs) and Applied New Drug Application (ANDAs) submitted to the Food and Drug Administration (FDA) contain the bioavailability data and in vitro dissolution data, that, together with chemistry, manufacturing and controls (CMC) data, characterizes the quality and performance of the drug product. For NDAs, in vitro dissolution specifications are generally obtained from batches that have been used in pivotal clinical and/or bioavailability, bioequivalence studies and for ANDAs, the in vitro dissolution specifications are based on the performance of acceptable bioequivalent batches of the drug products and CMC data. The FDA recommends dissolution testing of 12 dosage units for a variety of applications. Some of those applications are: ANDA submission to evaluate the difference factor (f1) and similarity factor (f2), Scale up Post Approval Control (SUPAC) conditions, development of an applicant’s dissolution method which is different from the FDA recommended method, modified release formulations, evaluation of alcohol-induced dose dumping, and manufacturing of multiple strengths of a dosage form from different blends. A dissolution tester capable of testing 12 dosages at one time may offer advantages with respect to time-saved, reduction in test variability and provide efficient use of resources such as water, electricity, man power and space. While a dissolution tester capable of testing 12 dosage forms may prove especially beneficial to a generic drug manufacturing company and a contract research laboratory, it may also prove to be useful to an ‘innovator’ pharmaceutical company.

Keywords: Automation, Dissolution, Laboratory Automation, Quality Control
Application Code: Pharmaceutical
Methodology Code: Other
Fiber-optic analysis for dissolution for a variety of dosage forms provides a range of benefits over traditional dissolution practices. Fiber-optic methods have been used for dual actives, high surfactant concentrations and unique excipients. Benefits include automation, sample manipulation, and use of complex media compositions.
This talk focuses on dissolution and drug release from suspension dosage forms that are dosed by the oral route of administration and include oral suspensions, suspensions for reconstitution and suspensions in capsules. The suspension typically consists of uniform particles that are readily suspended and easily dispersed. The most common reason to develop a suspension dosage form is limited aqueous solubility of the active pharmaceutical ingredient (API) at the dosage required. Another common reason to use suspensions is that they typically offer improved chemical stability compared to solutions. Suspensions are also used to achieve accurate weight-based dosing, which is limited for unit dosage forms such as tablets, by varying the volume of suspension delivered. Because suspensions are considered to be solid dosage forms, dissolution or drug release testing is required for these dosage forms as specified in USP general chapter <1088>. This talk will outline the factors that influence the dissolution for these formulations and what factors are unique to suspensions.

Keywords: Dissolution, Drugs, Pharmaceutical, Rheology
Application Code: Pharmaceutical
Methodology Code: Physical Measurements
Glutamate Modulation of Fast Acting Spontaneous Adenosine Release is Regulated Through the NMDA Receptor

Glutamate is an important excitatory neurotransmitter and is known to regulate learning, cognition, and memory. There are two types of glutamate receptors: ionotropic receptors and metabotropic receptors. Adenosine is a neuromodulator and regulates processes in conjunction with glutamate. Recently, we discovered fast acting spontaneous adenosine in the rat brain that is modulated by A1 receptor. Adenosine is released and cleared within a couple of seconds in the extracellular space and is not released through equilibrative nucleoside transporters. Here we examined the effect of ionotropic NMDA and AMPA glutamate receptors on the concentration, duration, and frequency of spontaneous transient adenosine. The NMDA antagonist, CPP (3-((R)-2-Carboxypiperazin-4-yl)-propyl-1-phosphoric acid), and the AMPA antagonist, NBQX (2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamine), were both administered to examine any effects on fast acting spontaneous adenosine. CPP increased the frequency of adenosine release events, while the agonist NMDA, did not decrease the frequency. The inter-event times between consecutive transients significantly decreased from 70.4 ± 4.5 seconds to 47.1 ± 3.0 seconds (p < 0.0001). Conversely, NBQX did not significantly change the inter-event times from 82.3 ± 5.7 seconds to 82.0 ± 6.0 seconds (p > 0.05). These results indicate that the frequency of spontaneous transient adenosine is regulated by NMDA glutamate receptors but not AMPA receptors. The feedback loop between transient adenosine and glutamate receptors indicates that these two molecules are tightly regulated and their mechanisms are connected in the brain.

Keywords: Bioanalytical, Electrochemistry, Microelectrode, Neurochemistry

Application Code: Bioanalytical
Methodology Code: Electrochemistry
Microfluidic paper-based analytical devices (mPADs) have been of increasing interest in point-of-care diagnostics and environmental monitoring due to paper’s inherent low cost, ease of modification, disposability, and ability to create flow without the need of external pumps or tubing. Although colorimetric detection has been the most common detection technique, electrochemistry offers a fast, sensitive, selective, inexpensive, and portable detection technique that can be readily adapted to paper, as our group first showed using screen-printed carbon electrodes. Here, two alternative mPADs with electrochemical detection (ePADs) will be discussed. Previously our group developed a colorimetric mPAD to detect enzymes produced by bacteria (Salmonella and E. coli) in both food and water samples. These same enzymes can also be used to produce electrochemically active products. Research to characterize these reactions and provide lower detection limits with shorter reaction times using electrochemical detection at carbon paste electrodes in ePADs will be discussed. While most reported ePADs have used carbon electrodes, microwires have only recently been incorporated. Microwires are available in a variety of compositions, highly conductive, well characterized, and can be easily cleaned and/or modified before being incorporated into the paper-based device. We have utilized microwires for the detection of analytes in a device design that produces quasi-steady flow, is capable of flow injection analysis and provides an increase in electrochemical signal in paper due to enhanced mass transport with flow and decreased electrode resistance.
Real time in vivo sensing of analytes in blood is the “holy grail” of sensor technology since, to date, intravascular chemical sensors often suffer from inaccurate results due to poor biocompatibility (clot formation, etc.). In addition, use of such devices poses significant risk of infection. Nitric oxide (NO) serves as a potent antithrombotic and antimicrobial agent in our bodies. In this presentation we will describe a new electrochemical NO generation approach to improve the biocompatibility and, thereby, the in vivo performance of an amperometric oxygen (PO2) sensing catheter. The new oxygen sensing catheter is fabricated using a dual lumen silicone rubber tubing, with one lumen dedicated to NO release from a copper complex mediated electrochemical reduction of nitrite ions at a stainless steel wire electrode, and the other lumen dedicated to PO2 sensing at a Pt wire electrode. The sensor has linear response to oxygen from 0% to 100% and this response is fully compatible with the electrochemical NO release chemistry. Electrochemical NO releasing PO2 catheters implanted within veins and arteries of animals (rabbits and pigs) for >8 h provide more accurate analytical results and less surface thrombus area compared to control devices without e-chem NO release. In vitro studies show that the catheters with e-chem NO release also exhibit greatly reduced surface attached bacteria, which would potentially decrease the risk of infection.
One of the key technical challenges for polymer electrolyte fuel-cell researchers is improving both the activity and stability of the oxygen reduction reaction (ORR) catalysts at the cathode. Pt-based alloys have shown great promise for achieving these targets. Herein, we report a new strategy for producing a highly active and stable class of PtCu catalysts with tunable compositions. Several unique nanoparticle catalysts were prepared by galvanically displacing a monolithic nanoporous Cu support with Pt, leading to a Pt-enriched PtCu shell over a PtCu core. It was observed that temperature during galvanic displacement is a key experimental parameter and a novel means to control catalyst particle size. This paper presents several modifications to the galvanic displacement process and describes the resulting enhancements in ORR activity (up to 2.1 mA/cm$^2$Pt) and stability relative to pure Pt.

Keywords: Electrochemistry, Electrodes, Energy, Fuels\Energy\Petrochemical
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Electrochemistry
**Abstract Text**

Rapid, real-time and on-site metal analysis is a useful diagnostic tool to treat trace metal contamination in the environment. We recently described an electrochemical method with high temporal resolution (100 ms) to detect copper and lead at carbon fiber microelectrodes (CFMs) using fast scan cyclic voltammetry (FSCV). Additionally, we characterized metal adsorption onto CFM surfaces as the fundamental mechanism of the rapid metal FSCV signal. In this work, we find that FSCV shows comparatively higher signals to Cu²⁺ than predicted by solution free metal content using geochemical, equilibrium models. Availability of free metal in complex matrices is a function of several intervening complexation reactions with different ligands. Equilibrium constants for these complexation reactions are an index of the strength of binding between metal and ligand. We explore the interactions between Cu²⁺ - CFM and Cu²⁺ - ligand equilibria using electrochemical, geochemical, and mathematical models. We find that different matrices do not affect the monolayer adsorption of copper on CFMs but that the Cu²⁺ - CFM equilibrium competes with solution equilibria to increase FSCV's sensitivity. We model the correlation between the FSCV signal, equilibrium constants for Cu²⁺ - ligand complexation, and free copper concentrations. This work is critical for further development of metal FSCV, particularly to increase sensitivity and selectivity.

**Keywords:** Electrochemistry, Environmental, Metals, Voltammetry

**Application Code:** Environmental

**Methodology Code:** Electrochemistry
Dopamine Transporter (DAT) Compensates for Impaired Serotonin Transporter (SERT) Function in the Small Intestine of Mice on a High Fat (HF) Diet

**Background:** Decreased transit time in diet-induced obesity (DIO) may result from increased gut mucosal 5-HT (serotonin) availability. 5-HT availability is dependent upon 5-HT uptake by the serotonin transporter (SERT) following its release from enterochromaffin (EC) cells. We determined if 5-HT availability was increased in the jejunum of control and high fat fed (HF) DIO mice.

**Methods:** 5-HT uptake was measured using amperometry in real time near the mucosal surface (i.e. near sites of release and uptake). Measurements from current approach curves were plotted as the ln(current) vs. electrode distance. A decrease in the slope of this plot described reduced 5-HT uptake. The SERT inhibitor fluoxetine (1 µmol L⁻¹) or the dopamine transporter (DAT) inhibitor GBR 12909 (0.1 µmol L⁻¹) was used to study changes in 5-HT uptake. Whole tissue 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) levels were measured using HPLC. SERT protein levels were measured using Western blotting.

**Results:** In HF jejunum, 5-HT uptake was insensitive to fluoxetine compared to controls suggesting SERT function was impaired. This was independent of SERT protein levels. GBR 12909 reduced 5-HT uptake in HF jejunum compared to controls suggesting DAT compensates for loss of SERT function. In agreement, whole tissue mucosal levels of 5-HIAA were similar in HF and control jejunum. Whole tissue mucosal 5-HT levels were not different in HF compared to control jejunum.

**Conclusions:** DAT compensates for loss of SERT function in DIO jejunum. Impaired SERT function increases 5-HT availability thereby potentiating 5-HT signaling to decrease transit time.

**Keywords:** Bioanalytical, Electrochemistry, Microelectrode, Neurochemistry

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
Our group has been developing a new electrochemical imaging tool termed fluorescence-enabled electrochemical microscopy (FEEM). The technique utilizes bipolar electrochemistry and fluorescence microscopy to simultaneously monitor electrochemical signal from very large (e.g. >10^6) arrays of parallel ultramicroelectrodes (UMEs), enabling the imaging of transient electrochemical processes with excellent spatial and temporal resolution. The central concept of FEEM is the use of a closed bipolar electrode to couple a conventional redox reaction of interest, such as the oxidation of dopamine, to a complimentary fluorogenic indicator reaction, such as the reduction of resazurin. The fluorescent product can be optically monitored via fluorescence microscopy to report the progress of the reaction of interest. This technique has several unique aspects, including the ability to drive the array with only one small voltage source (<1 V) and without any direct electrical connections to the array. Importantly, FEEM enables the study of non-fluorogenic redox reactions. Fluorogenic reduction and oxidation reactions have been incorporated into FEEM, meaning that both oxidizable and reducible species can be studied with the technique. Our initial studies on FEEM include imaging the diffusion of redox molecules from a micropipette and the electrocatalytic activity of platinum for H[sub]2[/sub]O[sub]2[/sub] oxidation, as well as the development of a suitable fluorogenic oxidation indicator reaction. Present imaging studies involve using FEEM to image the transient formation of diffusion layers of UMEs in both two and three dimensions. Work is also underway to understand and improve the spatial resolution of FEEM, develop and incorporate new fluorogenic indicator reactions, and improve the detection sensitivity. We hope to develop this technique into a tool that is useful and accessible to the broader analytical chemistry community and beyond.

Keywords: Electrochemistry, Fluorescence, Imaging, Microscopy
Application Code: Bioanalytical
Methodology Code: Electrochemistry
The protein [alpha]-hemolysin forms a pore with nano-meter dimensions in a lipid membrane through which single-stranded DNA (ssDNA) can translocate. As the DNA translocates through the pore, there is a momentary reduction of the current due to blockage of the ion flow. This system has been extensively studied as a potential method for rapid and cost-effective sequencing of ssDNA.

However, the interactions of double-stranded DNA (dsDNA) with [alpha]-hemolysin are less well-studied. While dsDNA can enter from the wider (vestibule) side of the pore, it does not translocate because its diameter is larger than the tightest (0.14 nm) constriction located at the center of the protein. With sufficient energy, dsDNA can unzip (denature) inside the vestibule, and the blocking current observed during dsDNA residence is due to its presence within the vestibule.

In this report, we describe a previously unrecognized sensing zone for dsDNA, in the vestibule of [alpha]-hemolysin that is sensitive to the presence of an abasic site (a missing base) within the DNA sequence. The presence and position of an abasic site can be detected over a 4-5 base (1.36 nm) range centered near to the entrance of the vestibule, with a sensitivity that is dependent on the precise position of the abasic site within the sequence. Temperature-dependent ion channel recordings that the detection of an abasic site in this newly-discovered sensing zone is possible because of a decrease in the activation energy required for transport of the electrolyte through the pore when an abasic site is in proximity to the constriction site. While previous efforts at nanopore DNA sequencing have focused on characterizing and sequencing ssDNA, the discovery of this new sensing zone offers exciting possibilities for characterizing and sequencing more biologically-relevant dsDNA instead.

Keywords: Bioanalytical, Biosensors, Biotechnology, Electrochemistry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
We present a method to identify and quantify methane using a hydrophobic ionic liquid (IL)–electrified metal electrode interface by electrochemical impedance spectroscopy. We investigated the mechanisms of the responses of the IL-electrified electrode interface to the exposure of methane and other interfering gases (H2, C6H12, SO2, NO, NO2, CO2, O2, H2O). Our results show that at low frequency the IL-electrified electrode interface shows a predominantly capacitive response. The IL-electrode double layer (EDL) was found to be the primary response layer while the transition zone and bulk region of the IL-electrode interface contribute little to the overall signal change. For recognition and quantification of methane using the Langmuir adsorption model and measurement of differential capacitance change, an optimum EDL interface structure was found to form at a specific DC bias potential. The cumulative results shown in this work suggest that an ideal IL-electrode interface can be formed by varying IL structure and applied DC bias electrode potential for a specific analyte and that the semi-ordered structure of IL-electrified interface can act as a recognition element for the sensitive and selective adsorption and detection of gaseous molecules.
Abstract Text

Extractive multi-pass cells have been used in the quantification of gas species present in flare vent gas systems using Fourier Transform Infrared Spectroscopy (FTIR). In a recent field test, FTIR has demonstrated an ability to match the performance capabilities of current gas chromatography based systems. FTIR’s ability to accurately quantify gas species present in flare vent gas systems is paramount to determining the Net Heating Value of the vent gas (NHVvg) in units of BTU/scf in order to meet regulatory requirements. However, in addition to FTIR, separate monitors and/or calculations are required to obtain concentration values for oxygen, hydrogen and nitrogen as these species cannot be directly measured using FTIR. The coupling of FTIR and Raman spectroscopy in a single extractive gas cell offers an intriguing combination of capabilities at a much lower total cost than current gas chromatography based systems. In addition to oxygen, hydrogen and nitrogen, Raman possesses the potential to detect and quantify many of the same gas species as FTIR albeit at higher mol % concentration levels. Results from the first operational prototype are presented here that demonstrate the capabilities of the Dual Function Gas Analyzer’s potential to simultaneously measure gas species of interest at both ppm levels for FTIR and mol % for Raman without additional monitors, calculations or specialized shelters.

Keywords: FTIR, Fuels\Energy\Petrochemical, Raman, Vibrational Spectroscopy
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Vibrational Spectroscopy
Raman spectroscopy can be beneficially applied to characterize oil shale for both its carbonaceous material content as well as the sedimentary rock consisting of clays, calcites, silicates, and titanium dioxides among other inorganic minerals. We have studied a variety of shale samples from different formations in the USA. We show that Raman spectroscopy is useful for characterizing the varieties of kerogen structure (chemical bonding and solid state) and the maturity of the carbonaceous matter, for identifying shales with different amounts of kerogen relative to inorganic mineral, and for differentiating polymorphs of naturally occurring inorganic oxides. Specifically, the Raman D and G band positions, widths and separations are a means by which the maturities of carbonaceous materials can be determined. Furthermore, we have found that Raman scattering excitation wavelength is a significant component to the proper interpretation of Raman data when comparing results obtained from different geological formations. Through Raman imaging, we find that inorganic minerals, including crystalline forms, can be imaged, identified and differentiated. Raman imaging complements optical microscopy by revealing the chemical and structural heterogeneity of carbonaceous and inorganic shale components that are not always revealed by optical microscopy.

Keywords: Fuels\Energy\Petrochemical, Microspectroscopy, Petroleum, Raman
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Molecular Spectroscopy
Photoelectrochemical Studies on Earth Abundant Pentanickel Polyoxometalates as Co-Catalysts for Water Oxidation

We report herein the photoelectrochemical studies on anatase TiO$_2$ photoanodes for solar water oxidation in the presence of a homogeneous pentanickel silicotungstate molecular water oxidation catalyst $\text{K}_{10}\text{H}_2\text{[Ni}_5\text{(OH)}_6\text{(OH}_2\text{)}_3\text{Si}_2\text{W}_{18}\text{O}_{66}\text{]}\cdot x\text{H}_2\text{O (Ni}_5\text{-POM).}$ Anatase TiO$_2$ thin films were prepared in-situ on fluorine-doped tin oxide (FTO) glass slides by a spin coating/thermal annealing technique, and XRD and SEM studies were employed for crystallographic and surface morphological characterization. Photoelectrochemical studies were performed in a quartz cell with a conventional three-electrode setup. A 150 W Xe lamp solar simulator equipped with an AM 1.5G filter was used as the light source for photocurrent and electrochemical impedance spectroscopic (EIS) measurements on FTO/TiO$_2$/Ni$_5$-POM system in the presence of the Ni$_5$-POM catalyst. In addition to a significant cathodic shift in the water oxidation potential, a maximum photocurrent of 0.2 mA/cm$^2$ was obtained from the FTO/TiO$_2$/Ni$_5$-POM system at 0.7 V vs Ag/AgCl (3 M KCl). These results could be attributed to the effect of catalyst on decreasing electron-hole recombination and facilitation of faster hole utilization kinetics on the semiconductor electrolyte interface upon light illumination, as indicated by EIS data. This is the first report on the use of water oxidation catalyst Ni$_5$-POM as a functional co-catalyst for photoelectrochemical water oxidation with semiconductor photoanodes. Moreover, the higher photocurrent characteristics of this system relative to a variety of previously reported POM based hybrid photoanodes has provoked further studies and possible employment in tandem photoelectrochemical water splitting strategies.

Financial support from the NSF CAREER Award (CHE-0955878, WJM) is gratefully acknowledged.

Keywords: Electrochemistry, Energy, Fuels\Energy\Petrochemical

Application Code: Fuels, Energy and Petrochemical

Methodology Code: Electrochemistry
Significant shale deposits exist in 22 states including the Northeast states of Ohio, Pennsylvania, and New York Utah and Wyoming in the West; and gas-producing states such as Texas, Louisiana, Arkansas and Mississippi. The use of horizontal drilling and hydraulic fracking of shale has catapulted the US into the leading producer of natural gas in 2013. Natural gas is bought or sold based on the quantity of energy delivered. The product of the concentration (determined by gas chromatography) and the heating value (BTU) determines the BTU content of the fuel. Although the natural gas from Marcelis shale is primarily methane, the composition can vary considerably from region to region. Shale gas streams can vary in composition from primarily CH4 to one that can contain heavier HC (to C6+) species. One does need flexibility in a GC and the GC301C with temperature programming does have it. The 301C has dual detectors (FID & TCD), packed and capillary column capability and temperature programming. It has an embedded PC and Windows 7.0 operating system with PeakWorks chromatography control software. It is a compact industrial gas chromatograph in a 19” rack mount or wall mount enclosure. Outputs include RS485, and 4-20 mA. It can be connected to the internet and can be controlled remotely. For natural gas, the methane content can vary from about 85 to 98 mole %, ethane varies from 1-7%, propane from 0.1 to 6%, nitrogen 0.2 to 6%, carbon dioxide from 0.1 to 1% with the balance of C4 and C5 hydrocarbons at trace levels. The exact composition can significantly change the BTU content. Methods can be stored for different compositions and changed remotely for additional flexibility in fracking operations.
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**Abstract Text**

The determination of bactericide efficacy in process drilling water has become a key process control problem faced by fracking drillers in areas where there is a limited supply of useable process water, limited disposal options for spent drilling water or concerns on bactericide concentration and the subsequent costs in the reuse water. The drilling project manager requires a rapid analytical test that measures the remaining active biocide in the drilling water. A bioanalytical process test utilizing aerobic and facultative anaerobic bacteria and measuring their active respiration has shown itself to be a sensitive quantifying process analytical test and producing accurate rapid results. This bioanalytical test is based on the “Standard Methods for the Examination of Water and Wastewater, 22nd Edition, Method 2710” utilizing an optical dissolved oxygen meter, constant temperature incubation optimized for biological growth at 40 °C, 15 mL sample vials with in-situ oxygen sensors, a nonspecific bacteria growth media and a bacteria source containing the biomass representative of that found in the environment. Samples prepared from a fracking water challenge matrix based on reference data from the petroleum industry and containing active biocide have shown a linear response between active biocide and bacteria mortality. This testing procedure will allow the drilling operations staff to make decisions on whether additional biocide should be added before drilling water reuse or if the spent water can be discharged to a biological treatment process for disposal. Funding provide by Baseline Industries.

**Keywords:** Bioanalytical, Fuels\Energy\Petrochemical, Process Analytical Chemistry, Water

**Application Code:** Fuels, Energy and Petrochemical

**Methodology Code:** Process Analytical Techniques
Advances in Energy Research: From Unconventional Fuels to Solar Energy

Side-Selective Modification of Photosynthetic Proteins for Highly Oriented Active Layers in Biological Solar Energy Conversion Applications

In recent decades many researchers have focused on the development of novel alternative energy solutions, with notable success in the arena of solar energy conversion. Progress has been somewhat limited based on expensive processing materials, processing costs, and generally low efficiencies. Nature, however, has already developed a comprehensive process of creating chemical energy from solar radiation. Photosystem I (PSI) is one of the enzymatic components in Photosynthesis that through a single photon absorption creates an electron-hole pair with nearly perfect quantum efficiency. This remarkable protein, once extracted from green plants, can be deposited onto electrode materials and used as a very inexpensive active layer in a simple photovoltaic cell. When found in nature, PSI functions with high efficiency by shuttling electrons unidirectionally across the thylakoid membrane bilayer from lumen to stroma. Thus, ex vivo, it becomes very important to orient the PSI complexes with high uniformity to minimize competing electrochemical reactions introduced by oppositely oriented proteins on a surface. In order to properly orient PSI we have developed a set of functionalization strategies, in which we selectively modify the exposed stromal side of this integral membrane protein prior to extraction, utilizing the thylakoid membrane bilayer as a natural barrier, leaving the luminal side of the protein completely unmodified after membrane lysis and purification. During a typical stromal-side functionalization procedure, a thiol-terminated ligand is conjugated to specific amino acids, which can activate our protein for preferential binding to a gold electrode. Through various spectroscopic and electrochemical analyses, we have demonstrated our control over the orientation of PSI. This work has enhanced the performance of PSI-based photovoltaics not only by aligning the proteins, but also through the direct “wiring” of the protein to the electrode.

Keywords: Electrochemistry, Electrode Surfaces, Energy, Nanotechnology
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Electrochemistry
It is imperative to understand biological variation in expression of metabolites due to genotype, environment, or both to assess the potential of metabolomics to supplement compositional analysis for substantial equivalence assessment of genetically modified crops. In the present study, we evaluated the biological variation of metabolites in various maize hybrids due to environment (growing location), genetic modification, trait, and interaction of the three. A total of 480 forage and 480 grain samples from 21 genetically diverse non-GMO DuPont Pioneer maize hybrids grown at eight North America locations were analyzed. GC/MS and LC/MS were used as complementary analytical platforms to detect a wide range of compound classes. A total of 166 and 137 metabolites were detected in forage and grain samples by GC/MS, while 1341 and 635 metabolites were detected in forage and grain samples by using LC/MS positive mode and negative mode combined. Univariate and multivariate statistical analyses were utilized to investigate the main effects and interaction of environment, genotype and traits on the maize metabolome. The results revealed that the environment had far more impact compared to genotypes or traits. In forage samples, the environment affected 50% to 80% of the metabolites, while less than 7% were affected by genotypes. In grain samples, the environment affected 50% to 90% of the metabolites, whereas less than 20% were affected by genetic backgrounds. Additionally, the effects of drought and disease resistant traits were studied within each location, and less than 10% of the metabolites were affected by these traits. Overall, the results of this comprehensive study support and extend previous findings about the environmental and genetic perturbations on the maize metabolome.
In the oven drying procedure to measure moisture content in cotton by drying in ambient air at 105 – 110°C, the weight of the lost volatiles is the measure of moisture content, all of which is incorrectly attributed to water. The origin of these biases is incomplete drying and weight change due to side reactions in opposing directions. The standard test method (ASTM D7785-12) for water in white lint cotton by oven evaporation combined with volumetric Karl Fischer Titration (KFT) is specific for total water (free and bound). A test specimen is sealed in a glass vial, placed into a micro oven and dry nitrogen transports the water vapor from the fibers into a titration cell. The amount of water is determined by volumetric titration with Karl Fischer reagent; the end point indicated by electrodes that measure a sharp change in potential when the iodine in the reagent is reduced by sulfur dioxide in the presence of water. The objective of the present study was to extend the ASTM method to measure total water in three other cellulosic fibers: brown cotton, flax and rayon. Method validation involved checking for residual water in a specimen by NIR spectra taken through the bottom of the vial and Karl Fischer selectivity to water over interfering substances. Relative water concentration in the cottons (white and colored) and non-cottons (flax and rayon) will be discussed. Sample conditioning in a glove box placed in the isothermal textile testing room (21 ± 1°C) to control humidity within 65 ± 0.5%, by the use of a saturated aqueous salt solution, provided the best precision. ASTM D7785 was successfully applied to measuring the level of water in all cellulosic materials. Comparative information on the water content of different types of fibers conditioned side-by-side is highly desirable since their physical properties are significantly affected by moisture. Variations in the amount of water present, or its regain, affect the mass of dry fiber in a lot of material.

Keywords: Agricultural, Reference Material, Titration, Water
Application Code: Agriculture
Methodology Code: Chemical Methods
Explants treatment with growth regulators is a widely adopted procedure in vegetative plant propagation, being more crucial in difficult-to-root species or cultivars where otherwise the adventitious rooting process may never occur at all. Several studies have suggested that differences in rooting ability of cuttings could be attributed to the differences in uptake or metabolism of the absorbed auxins. Hence, it is important to monitor the evolution of endogenous levels of these compounds in auxin-treated cuttings, preferentially as a routine practice. Gas chromatography/mass spectrometry (GC/MS) has been widely used for the analysis of plant growth regulators and, despite the significant progress achieved in analytical methods for auxins quantification, sample preparation is still the bottleneck of the process. Microextraction techniques such as dispersive liquid-liquid microextraction (DLLME) offer several advantages like low solvent consumption and fast sample extraction. However, since auxins are not naturally volatile, their analysis by GC/MS is dependent on previous derivatization, which so far has been done using time-consuming conventional silylation methods. Microwave derivatization (MAD) takes advantage of the dielectric heating effect to greatly reduce derivatization time and improve reaction efficiency. Here we report the development of a new method for the quantification of free auxins in Olea europaea L. samples, using DLLME and MAD followed by GC/MS analysis. Optimization of DLLME conditions, as well as MAD conditions, is described. The proposed method proved to be substantially faster than other alternatives, and has already been applied to real samples of semi-hardwood cuttings and microcuttings of olive, which results are presented.

Acknowledgements:
This work was supported by FEDER funds through COMPETE, by national FCT (Fundaçao para a Ciencia e a Tecnologia) funds, under the projects PTDC/AGR-AAM/103377/2008 and Pest-C/AGR/UI0115/2011 and by the POR-Alentejo (InAcento) Operation ALENT-07-0262-FEDER-001871. The first author would like to further acknowledge support by FCT’s Doctoral Grant No. SFRH/BD/80513/2011, the Department of Energy (DOE) grant number DE-FG02-93ER20097 for the Center for Plant and Microbial Complex Carbohydrates at the CCRC, as well as Parastoo Azadi at the Complex Carbohydrate Research Center for its gracious support in her research while in the United States.

Keywords: Agricultural, Derivatization, Extraction, Optimization
Application Code: Agriculture
Methodology Code: Gas Chromatography/Mass Spectrometry
Waste extracts of Jamaican sorrel ([i]Hibiscus sabdariffa[/i], S) and citrus ([i]Citrus sinensis[/i], C) were evaluated for their potential commercial applicability. Pectin (0.84 % C; 0.48 % S), crude fiber (3.94 % C, 4.16 % S), ash (3.21 % C; 4.37 % S) and crude fat (2.65 % C; 3.15 % S) of the extracts were assessed. Fatty acid analyses utilizing gas chromatography/mass spectrometry revealed that the extracts are a source of omega 6 (19.90 % C; 25.37 % S) and omega 9 fatty acids (18.24 % C; 20.82 % S). Palmitic acid (32.94 % C; 42.45 % S) and stearic acid (7.21 % C; 7.64 % S) were also identified. Nuclear magnetic resonance spectroscopy showed characteristic triglyceride signals, a doublet of doublet (4.15; 4.30) and a multiplet (5.35) which are due to the protons on the glyceride backbone. Elemental analysis by atomic absorption spectroscopy revealed higher levels of iron (69.76 ppm S; 8.36 ppm C) and zinc (367.13 ppm S; 5.00 ppm C) in sorrel extracts. The antioxidant activity of extracts was determined by the 2,2-diphenyl-1-picrylhyrazyl (DPPH) assay and the total phenolics by the Folin-Ciocalteu assay. Total phenolics was higher in citrus extracts. Conversion of cellulosic biomass to usable value added products presents an opportunity for agro processing industries to reduce the quantity of waste effluent which arises from the agriculture industry. Sorrel and citrus extracts may be considered for use in nutraceuticals.
Detection of Weapons of Mass Destruction

Chemical Warfare Agent Detection and Quantification with a Person Portable GC/MS System

Fast, low level detection of chemical warfare agents (CWAs) in the field is needed to keep both military personnel and civilians safe during and after chemical threats and incidents. Ideal detectors need to be fast, rugged, and person portable, with the ability to detect, identify, and quantify complex mixtures containing chemical warfare agents and related chemicals. The HAPSITE ER, the only person portable GC/MS, was designed for the analysis of volatile and semi-volatile organic chemicals in air. The higher boiling points and increased chemical activity of organophosphate CWAs required modifications to the GC/MS system to increase detection. Modifications include increased sample pathway deactivation, higher temperature sample pathway components, and longer sample desorption times. Maintaining battery-operated portability requires lower than ideal component temperature settings, but by prioritizing critical portions of the sample pathway, detection limits of the CWAs tested were significantly reduced with improved reproducibility. Effects of system modifications were monitored using tributyl phosphate, a CWA simulant chemical. Testing with actual CWAs was carried out by a third party surety lab in the United States. Detection limits were found to meet or exceed IDLH (Immediately Dangerous to Life and Health) limits for airborne exposure.

Keywords: Environmental/Air, Gas Chromatography/Mass Spectrometry, Portable Instruments
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography/Mass Spectrometry
## Abstract

The utility of hyperspectral imaging (HSI) passive chemical detection that utilizes wide field, standoff screening continues to advance for detection applications. With a drive for reduced size, weight, and power (SWaP), near real-time detection capabilities, and improved sensitivity, developing a portable sensing platform that is robust and user-friendly increases the detection capabilities of the sensor.

ChemImage Sensor Systems has developed a portable HSI sensor, called VeroVision[circumflex O], for real-time, wide area surveillance and standoff detection of explosives, chemical threats, and narcotics. Utilizing liquid crystal tunable filter technology, VeroVision[circumflex O] provides wide-field SWIR imagery combined with an intuitive user interface that produces automated detections and a real-time display of threats with a built in library of threat signatures that is easily updated, allowing for the input of additional materials of interest. Unlike existing detection technologies that often require close proximity for sensing, VeroVision[circumflex O] allows the individual operator to detect threats from a safe distance.

This presentation will provide an overview of shortwave infrared spectroscopy, focus on the design and operation of the sensor, and discuss specific use scenarios that VeroVision[circumflex O] can be applied to, such as explosive detection or narcotic identification.

### Keywords
- Infrared and Raman
- Instrumentation
- Portable Instruments
- Vibrational Spectroscopy

### Application Code
- Homeland Security/Forensics

### Methodology Code
- Vibrational Spectroscopy
The goal of this research was to investigate trace metal composition across different sources of inorganic pool chlorine (calcium hypochlorite) both as a precursor compound and as residues from a hypergolic mixture. This is a new forensic signature system for the attribution of unknown chemical residues recovered from arson-related crime scenes.

Three different sources of calcium hypochlorite were combined with automotive brake fluid to create a self-igniting mixture. The residues were extracted with nitric acid and analyzed with Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) for 24 different elements. Multivariate chemical profiles were then analyzed using Discriminant Function Analysis (DFA).

ICP profiles showed that each hypochlorite source exhibited distinct variation in the presence and relative abundance of certain elements that was consistent in both pre- and post-reaction residues. For example Aluminum was enriched in the UC sample group (~40ppm vs. <7ppm) while Boron was more concentrated in the SS group (~2ppm compared to the other sources (<0.09ppm). Error rates for all measured elements were less than 0.05ppm. DFA of ICP profiles revealed robust separation among all three hypochlorite sample groups along the first two functions. Function coefficients indicated that Al, Cr, Cu, K, Ni, and Sr contributed most to the variation among groups. Overall, this work suggests that trace metal variation can be used to differentiate each source of calcium hypochlorite and that multivariate metal profiles may be a useful signature for comparing hypergolic residues to precursor compounds or different residue samples to each other during an investigation.

Keywords: Chemometrics, Forensic Chemistry, ICP, Trace Analysis
Application Code: Homeland Security/Forensics
Methodology Code: Other
Binary explosive mixtures are commonly found in improvised explosive devices (IEDs) which have become increasingly commonplace in the Middle East. The components of such mixtures often have common, innocuous uses independent from each other and thus are only of concern when they are combined. Binary explosives require an oxidizer and a fuel component. Examples of oxidizers include ammonium nitrate (AN) and potassium chlorate (PC), while examples of fuels include, but are not limited to, sugar, aluminum powder, and fuel oil. Detection protocols often focus on the detection or sensing of the oxidizer alone. Canines, for example, are often trained on solely AN, though recent canine evaluations have shown that the canines perform better when trained to the mixed components.

Hazards associated with binary explosives mixtures often limit or preclude analytical research and other testing using the mixtures themselves. Through this research a novel device was designed that safely segregates the binary explosive components, but provides a mixed volatile signature. For this reason, it is important to establish differences, if any, between explosive components and component mixtures. Qualitative and quantitative differences in the headspace of the individual fuel and oxidizer components and the mixtures were compared, with the age of the mixture being explored as well. Vapor analysis was carried out by GC/MS with whole air sampling with online cold trapping, as well as solid phase micro-extraction (SPME). Ammonia, derived from the dissociation of ammonium nitrate in ambient humidity, was collected by passive sampling onto sorbent disks with analysis by ion chromatography. Results showed minimal differences between individual explosive components and fresh mixtures.

Keywords: Forensic Chemistry, Volatile Organic Compounds
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography/Mass Spectrometry
Detection of Weapons of Mass Destruction

UV Resonance Raman Study of the Photochemistry of Trinitrotoluene (TNT) and Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

We examined the 229 nm deep ultraviolet resonance Raman spectra (DUVRR) of solution and solid state trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and their solution and solid state photochemistry. TNT photodegrades with a quantum yield of ~ 0.015. The initial photoproducts of TNT photolysis have similar enhancement of the –NO2 stretching vibrations as TNT, resulting in DUVRR spectra that are very similar to that of pure TNT. The photoproduct bands result in TNT-like DUVRR spectra even after TNT is completely photolyzed. These ultraviolet resonance Raman (UVRR) bands enable DUVRR of trace and standoff detection of TNT. RDX rapidly photodegrades with a high performance liquid chromatography (HPLC) measured solution quantum yield of ~ 0.35. During the photolysis of RDX, new spectral features evolve indicating photoproduct formation. A stable DUVRR spectrum is seen after long irradiation times that enable standoff detection of RDX. Nitrate is a photoproduct that forms in the solution state photolysis of RDX and is a signature for detection of RDX even after photolysis.

The structures of the initial TNT photoproducts were determined through liquid chromatography mass spectrometry (LCMS) and tandem mass spectrometry (MS/MS). The solution and solid state photochemistry of TNT shows similar DUVRR spectra and initial photoproducts. The major solution state initial RDX photoproducts were determined with high performance liquid chromatography-high resolution mass spectrometry (HPLC-HRMS) and gas chromatography mass spectrometry (GCMS). We used X-ray photoelectron spectroscopy (XPS) to determine photoproducts formed during solid state RDX photolysis. This work was funded by the Office of Naval Research (ONR) N00014-12-1-0021 contract.

Keywords: Detection, Raman, Spectroscopy, Vibrational Spectroscopy
Application Code: Homeland Security/Forensics
Methodology Code: Vibrational Spectroscopy
Ion mobility spectrometers are already in routine use e.g. as handheld systems for military use (chemical warfare agents) or fire fighters (hazardous substances) and at airports for the detection of drugs of abuse and explosives using wipe tests and thermal desorption units. However, all those instruments have in common that they are optimised for particular compound groups and are not selective enough to cover a broad range of applications. We demonstrated that using rapid gas-chromatographic pre-separation combined with an internal gas-supply, a GC ion mobility spectrometer can be applied as a mobile system with the full analytical performance. Particular applications are searching for trapped victims during urban search and rescue operations but the detection of hidden persons as well, the detection of the consumption of drugs of abuse e.g. for traffic control and the detection of explosives or other hazardous substances e.g. in room air. The use of pattern data bases and suitable algorithms enable rapid on-site data evaluation.

Keywords: Drugs, Environmental/Biological Samples, Forensic Chemistry, Portable Instruments
Application Code: Homeland Security/Forensics
Methodology Code: Chemical Methods
Can a Neurological Disease Begin in the Bloodstream? Remarkable Results from Quantitative Analyses of Blood from People with Multiple Sclerosis

Multiple Sclerosis (MS) is a disease of the central nervous system where the myelin sheath covering the axons of nerve cells becomes damaged. There is currently no cure for MS and the mechanisms of action for current therapies are unknown. In some cases, the diagnosis of the disease may require multiple MRIs over a period of months to years to diagnose. In an attempt to better-understand the mechanisms involved in demyelination, our group has discovered that, when exposed to mechanical deformation, red blood cells (RBCs) from MS patients release nearly 2-3 fold more ATP (~385 nM) than control RBCs (~190 nM). Importantly, ATP is a stimulus of NO, a molecule known to break down the blood brain barrier, a classic feature of MS. Recently, we have also shown that physiological steroids, such as estrogens, can decrease the NO stimulated by RBCs. In this work we use a 3D-printed microfluidic device to mimic in vivo conditions to better understand the mechanisms of action of common MS steroid therapeutics. These measurements are performed using both chemiluminescence (for ATP) and fluorescence (for NO) detection in different wells on the 3D-printed device. Other data to be presented, from 40 MS blood samples, may provide insight for these high ATP release values. Our device allows for multiple steroids or multiple concentrations of the same steroid to be measured in parallel for high-throughput results, while still maintaining flow-based measurements and communication between a circulating blood stream and the baso-lateral side of an endothelium.

Keywords: Biological Samples, Clinical/Toxicology, Drugs, Lab-on-a-Chip/Microfluidics
Application Code: Clinical/Toxicology
Methodology Code: Microfluidics/Lab-on-a-Chip
The ability to select ligands that specifically bind a target of interest can aid in the development of drugs, clinical assays, and more. Some binding events, however, result in only minor perturbations to the inherent physicochemical properties of ligand or target, making separation and detection of bound and free species a challenge, especially for restricted sample sizes and trace sample constituents. The goal of this work is to overcome these challenges by developing versatile and highly efficient capillary transient isotachophoresis (cITP) methods suitable for the selection of candidate ligands ranging from small macrocycles to large aptamers, acting on various classes of targets, ranging from proteins to microbes. By employing background electrolyte modifiers such as dilute, soluble polymer to facilitate sample zone focusing (by the method of Polymer Enhanced Capillary Transient Isotachophoresis, PectI), and/or SYBR Gold nucleic acid stain for on-column fluorescent labeling of samples, we have been able to resolve, detect, and collect ligand-bound target fractions for subsequent characterization. In preliminary studies, the average enrichment of thrombin-binding aptamer from a random DNA library was almost 40-fold after a single cITP run, opening the door to advanced method development for the discovery of new ligands from a 13,824 member library of DNA-encoded small molecules against clinically relevant Src tyrosine kinase, and from an 88-base random DNA library against model microbial targets [Escherichia coli] and [Bacillus subtilis]. These selection experiments are fast and highly efficient, and show promise for future application to microbial pathogens, disease biomarkers, and other targets of relevance to human and environmental health and safety.

Keywords: Bioanalytical, Capillary Electrophoresis, Drug Discovery, Method Development
Application Code: Drug Discovery
Methodology Code: Capillary Electrophoresis
Synthetic unmethylated cytosine-guanine (CpG) oligodeoxynucleotides (ODNs) are immunostimulatory motifs that have shown tremendous promise as vaccines or adjuvants for treatment of cancers and infectious diseases. For effective immunotherapy, it is necessary to efficiently deliver these nucleic acid therapeutics intracellularly into target immune cells while protecting them from nuclease degradation. We have developed novel immuno-nanoflowers (NFs), which are self-assembled from long DNA encoded with tandem CpG through rolling circle replication (RCR), for efficient CpG delivery and protection from nuclease degradation. In a model of macrophage-like cells, the CpG-NFs proved to be potent immunostimulators by triggering the proliferation of these immune cells, which, in turn, secreted immunostimulatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-10 (IL-10). These results demonstrate the ability of CpG-NFs to induce cancer cell apoptosis and necrosis through their immunostimulatory properties.
Cystic Fibrosis (CF) is an autosomal recessive genetic disorder that disrupts the cystic fibrosis transmembrane conductance regulator protein (CFTR). An important chloride ion channel, CFTR is present in numerous cell types, including pancreatic beta-cells, red blood cells (RBCs), and neutrophils. Perhaps not surprisingly, patients with CF have complications associated with diabetes, blood flow, and immune response. In this presentation, we will present data showing a possible link between these three body systems (endocrine, circulatory, and immune). Specifically, we will present results quantifying the amount of C-peptide ($0.76 \pm 0.16 \text{ pmol/million cells}$) and zinc ($2.5 \pm 0.3 \text{ pmol/million cells}$), two secretogues from beta-cells, bound by control human neutrophils. C-peptide, when secreted from the endocrine system, has known effects on the circulation, including binding to RBCs and stimulating increases in blood flow. However, its downstream effects on the immune system are not understood. We will describe the design and use of a rugged, reusable 3D-printed microfluidic device to determine the role of C-peptide and Zn$^{2+}$ on neutrophil adhesion and chemotaxis. This novel fluidic device enables exquisite control of included cells and tissues and allows for high-throughput analysis of different conditions, such as chemoattractant concentration and neutrophil stimuli, in parallel.

**Keywords:** Biological Samples, Biomedical, Lab-on-a-Chip/Microfluidics, Microscopy

**Application Code:** Biomedical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
LC-MSn screening of “sexual performance enhancing” dietary supplements resulted in the discovery of several analogs of approved erectile dysfunction drugs not previously detected by the Food and Drug Administration’s Forensic Chemistry Center. Additionally, many recently analyzed products have contained multiple active pharmaceutical ingredients and/or analogs, which have been identified by liquid chromatography with mass spectral detection (LC-MS) and assayed by high performance liquid chromatography with ultraviolet detection (HPLC-UV). Here we report the recent trends observed in the detection of PDE-5 inhibitors and their analogs contained in dietary supplements, including the first identification of a compound that structurally resembled tadalafil. One new analog was isolated from the supplement matrix using HPLC with fraction collection, and was further characterized using nuclear magnetic resonance (NMR) and LC-MS with accurate mass determination. The analog had an accurate mass of $[\text{m/z}] = 420.15614$ (error is 1.77235 ppm) for the protonated species $[\text{M+H}]^+$, corresponding to a molecular formula of $\text{C}_{23}\text{H}_{22}\text{N}_3\text{O}_5$. Mass spectral fragmentation data suggested that the modification occurred in place of the $-\text{CH}_3$ located on the pyrazinopyridoindole-1,4-dione of tadalafil. NMR was utilized to further elucidate the configuration of the substitution. The analysis indicated that the moiety is a $-\text{CH}_2\text{CH}_2\text{OH}$, hydroxyethyl group. The new analog has been named 2-hydroxyethylnortadalafil.
**Session Title**: Drug Discovery, Pharmaceuticals and Biomedical Analysis  
**Abstract Title**: Quantitative Enumeration of Viable Bacteria after Antibiotic Dosing Using a 3D Printed Device

**Primary Author**: Jayda L. Erkal  
**Co-Author(s)**: Dana M. Spence, Sarah Y. Lockwood

### Abstract Text

The rise in antibiotic resistance has resulted in a need for high throughput platforms that enable generation of dosing profiles and measurement of bacteria response. Here, a 3D-printed device was used as a platform to control dosing profiles to bacteria (Escherichia coli). The 3D-printed device has 6 channels, each with dimensions of 1 mm x 1 mm x 0.5 mm. Each channel holds a transwell membrane insert (polyester membrane, 0.4 μm pore size) that is used to house the bacteria. E. coli was chosen as the model bacteria, while Levofoxacin was chosen as the antibiotic. E. coli was grown in lysogeny broth with shaking overnight. After approximately 14 hours, the cells were centrifuged and rinsed with albumin free physiological salt solution (BSA-free-PSS) 3 times and adjusted to a concentration of 10^8 cells/mL. Samples were loaded into the transwell inserts and antibiotic was pumped through each channel at 10 uL/min using syringe pumps. Exposure profiles were monitored using LC/MS and were controlled by variation in the concentration of antibiotic flowing in the channel and pumping time. Preliminary fluorescence data using 2-color staining (SYTO9 and propidium iodide) indicates that after dosing with levofoxcin over a period of 3 days, 87% ± 13% (day 1), 17% ± 19% (day 2), and -1% ± 2% (day 3) bacteria remained live after dosing. Bacteria viability, determined using the 2-color system and flow cytometric methods, in response to various dose curves will be discussed.

**Keywords**: Biotechnology, Drugs, Lab-on-a-Chip/Microfluidics, Pharmaceutical

**Application Code**: Pharmaceutical

**Methodology Code**: Microfluidics/Lab-on-a-Chip
Bench top NMR has proven itself to be an interesting new tool for obtaining quick, easy, and inexpensive NMR spectra for research and instructional purposes. It can also be used to attain spectral profiles on pure samples of drugs that produce “legal highs”. This work attempts to create an NMR database of synthetic compounds known to be in use recreationally, or analogs thereof. Such a library would be useful to those working in crime laboratories to identify unknown chemical compounds found at crime scenes. This test is more accurate than a color indicator and is easier to administer than a traditional mass spectrometric (MS) analysis. A single test requires only twenty minutes and is very cost effective.

Major classes of compounds tested include psychedelics, cathinones, and cannabinoids. Standard samples were provided by Cayman Chemical (Ann Arbor, MI) and street samples were provided by the Pennsylvania State Police (Harrisburg, PA). These street samples were mostly mixtures of compounds most notably XLR-11, PB-22, and AB-Fubinaca. Compounds were dissolved in the appropriate deuterated solvent and centrifuged to insure the compounds completely dissolved.

The standards’ spectra are comparable to those of the samples. An interactive library will be compiled so a drug profile can be quickly compared against a library and a presumptive identity can be established. This method will not be meant to definitively identify an unknown compound but it can aid in rapid presumptive analysis so that follow-up analyses are more targeted.

NMR will consistently give a general profile of a given compound. If there is no discrepancy in shimming and solvent choice, NMR can be used to compare spectral similarities among unknown compounds. A quick and easy presumptive test for drugs, provided by the bench top NMR, can be useful to save time on a more in depth instrument and accurately identify at least the class of compound of an known substance.

**Keywords:** Drugs, Forensics, NMR

**Application Code:** Drug Discovery

**Methodology Code:** Magnetic Resonance
Chirality is an important characteristic of many pharmaceutical drugs. Often, one of the enantiomers exhibits specific pharmacological and biological function, or even toxicity. Thus, the information about chiral purity is needed for better evaluation of the drug. To facilitate this analysis, we developed a new class of chiral selectors that could be used as additive in capillary electrophoresis (CE) for the separation of enantiomers: sulfopropyl ether-[alpha]-cyclodextrin polymer (SPE-[alpha]-CDP), which was a novel, highly-hydrophilic, negative charged chiral additive.

SPE-[alpha]-CDP could synthesize through a simple and efficient procedure and it proven to have statistic uniformity through indirect UV detection. Elemental analysis and blue tetrazolium detection had been used to characterize the degree of sulfonation and the content of [alpha]-CD, respectively. By using this chiral selector, model chiral drugs of weak acid, weak base and neutral drugs had been separated with different separation conditions including the concentration of SPE-[alpha]-CDP and buffer, pH of buffer, and the applied voltage. Comparing to the non-polymerized sulfopropyl ether-[alpha]-cyclodextrin (SPE-[alpha]-CD), SPE-[alpha]-CDP gave a much lower operation current under similar conditions. The polymerization made SPE-[alpha]-CDP readily recyclable. The recycled products were proved to have similar resolution with original ones through separating the same analytes.

Keywords: Capillary Electrophoresis, Chiral, Chiral Separations, Cyclodextrin
Application Code: Pharmaceutical
Methodology Code: Capillary Electrophoresis
Electrochemistry - Electrodes

Novel Electrode Material for Efficient Ethanol Oxidation Using Tunable, Three-Dimensional Poly (amic) Acid (PAA)

Direct alcohol fuel cells (DAFCs), particularly those utilizing ethanol as a fuel, have attracted more attention as alternative energy source[1]. Direct ethanol fuel cells can work at low temperature, possess high theoretical mass energy density, and are environmentally friendly. Ethanol Oxidation Reaction (EOR) can be operated both in acidic or alkaline solutions. Fundamental studies of ethanol electro-oxidation in acidic media have been mostly performed on platinum. Palladium, on the other hand has been less studied in acidic media due its relatively low performance[2-4]. An efficient electrode for a fuel cell must be conductive, hydrophobic, requires high surface areas and high porosity to enable mass transport of H+ as well as be corrosion-resistant. Numerous materials are widely studied including carbon nanotubes, carbon black, and graphene. We hereby present the use of conjugated poly(amic) acid (PAA) [5-8] as novel materials for EOR.

Keywords: Electrochemistry, Electrodes, Energy, Environmental
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Electrochemistry
**Abstract Text**

Biological analysis, environmental monitoring, food safety and disease diagnosis require sensitive, low-cost and multiplex assay. Microelectrode array (MEA) could meet these requirements and has been receiving significant attention because of its advantages such as higher sensitivity (versus macroelectrode) benefited from the enhanced mass transportation, improved signal-to-noise ratio and larger current response (versus microelectrode). So far, the fabrication of regular MEA has been accomplished mainly by depositing an insulating layer on a macroelectrode surface and then making patterned holes in this layer with photolithography, which were often prohibitively expensive and time-consuming. Soft lithography has been proved to be a rapid and cost-effective method to form and transfer patterns and structures. Our group commit to develop various soft lithography for fabricating MEA. We reproducibly fabricated regular Au band MEA with hydrogel etching and proposed a new technique to fabricate reduced graphene oxide (rGO)-based MEA with microtransfer molding. The developed MEA showed a lower detection limit and a larger current density for the detection of biological compounds, such as H2O2, dopamine, as compared with the macroscopic electrode.

ACKNOWLEDGMENT: We gratefully acknowledge the financial support from National Natural Science Foundation of China (Grant 21175151)

**REFERENCES**


**Keywords:** Electrochemistry

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
Electrochemistry - Electrodes

Examining Surface Chemistry of Carbon-Fiber Microelectrodes during the Application of a Dynamic Potential Using Raman Spectroscopy

Carbon-fiber microelectrodes have proven to be an ideal sensor for studies in vivo. When combined with background-subtracted fast-scan cyclic voltammetry (FSCV), they provide a highly sensitive platform for working in biological tissue. The performance of carbon-fiber electrodes is significantly improved by electrochemically oxidizing the surface of the electrode. Thus, it is common to electrochemically condition a carbon-fiber microelectrode before an experiment by repeatedly cycling to 1.0 V or greater; however, the fundamental mechanism underlying the resulting signal enhancement has not been fully resolved. We have used a combination of Raman spectroscopy and electrochemical methods to characterize electrochemically oxidized carbon surfaces. Spectroscopic measurements were taken while polished carbon-fiber electrodes were conditioned using a variety of dynamic waveforms and static potentials. The disorder associated with the carbon surface was correlated to electrochemical sensitivity for the detection of dopamine and ascorbic acid using FSCV. The work herein provides unique insight into the chemistry of a dynamic surface that will prove invaluable for future studies seeking to improve detection performance for specific analytes.

Funding provided by: North Carolina State University Department of Chemistry
NCSU Research and Innovation Seed Funding Program
NSF CAREER CHE7151264

Keywords: Electrochemistry, Microelectrode, Raman, Surface Analysis
Tetrahedral amorphous carbon (ta-C) is a type of diamond-like carbon that exhibits properties closely resembling those of diamond including high hardness, wear resistance, optical transparency, corrosion resistance, as well as, electrical conductivity. Additionally, doping ta-C with different concentrations of nitrogen (ta-C:N) during film growth is a common way to control these properties. Knowledge of the physical, chemical and electrical properties of ta-C:N films is necessary to understand the mechanisms and kinetics of various redox systems. This presentation will focus on describing the background voltammetric response and voltammetric response of ta-C:N films, doped with various amounts of nitrogen, to behaviorally-different redox systems in aqueous media. The results will show that these carbon materials are particularly good for electroanalysis.

Additionally, ta-C has been used to inhibit protect various metals from both corrosion and wear. Detailed studies of the corrosion inhibition mechanism of these coating on various steels have not been fully conducted. Our research is addressing this knowledge gap and the presentation will highlight some of our recent findings on the degree of corrosion inhibition the coatings provide on steel. Specifically, the ability of ta-C and ta-C:N coatings to protect steel from corrosion in accelerated corrosive testing environments.
In spite of their significance in determining battery performance, a comprehensive understanding of how local surface (de)activation and site-specific differential reactivity impacts the dynamic ion-transfer capabilities of ion-battery anode interfaces has yet to be fully elucidated. New in situ analytical tools are required to address present and future battery challenges by incorporating versatile platforms capable of analyzing alkaline ions at interfacial nano-structures in aqueous and organic solvents.

Here, we introduce two approaches for the imaging of ionic reactivity based on scanning electrochemical microscopy using liquid probes. In the first approach, Hg hemispheres supported on micro- and nano-probes were used for the detection of ionic fluxes of Na\(^{+}\), Li\(^{+}\) and K\(^{+}\) in propylene carbonate over operating Au and C electrodes. In the second approach, the ion transfer across immiscible electrolyte solutions (ITIES) was used at the aqueous/1,2-dichloroethane interface using suitable ionophores to detect Li\(^{+}\) and Na\(^{+}\) fluxes in aqueous media over operating TiO\(_2\) electrodes. In both cases, the probe potential provided chemical specificity while the limiting current for ion ingress into the liquid probes allowed the measurement ionic fluxes with excellent stability and linearity for concentrations in the mM and sub-mM range. SECM electrochemical maps with sub-[micro]m spatial resolution probed quantitatively the alkaline ionic reactivity of controlled-defects generated by focused ion beam on C and Au, as well as surface-segregated redox domains on TiO\(_2\). This technique represents an unprecedented step in the analysis of anode ion insertion mechanisms, accessing aspects of surface reactivity that are lost during averaging in conventional electrochemical methods.
Kinetic Size-Spectra of Gas Molecules at an Ionic Liquid (IL)-Metal Interface and Its Application for Highly Selective Gas Sensing

Ionic liquids (ILs) were widely used as nonvolatile solvents and electrolytes. However, their properties resembling the crystalline solids have not been fully recognized. In this report, we show that an IL-electrified electrode interface has a potential-driven crystalline ordering with cavities enabling selective adsorption of a specific size of gas molecule. With this unique interface, a kinetic size spectroscopy related to the molecule dipole moment and kinetic diameter (KD) of adsorbate molecules was established for gas identification with 0.1 Å of KD resolution.

Keywords: Adsorption, Electrochemistry, Electrode Surfaces, Environmental/Air
Application Code: Environmental
Methodology Code: Electrochemistry
Abstract Text

Electrochemical aptamer-based (E-AB) sensors are specific, selective, sensitive, and applicable to the detection of a variety of targets. The specificity is afforded by an electrode bound RNA or DNA recognition element, or aptamer. A redox tag on the distal end of the aptamer enables the signaling of these sensors. Signaling results from target-induced conformational changes or flexibility of the aptamer probe. These changes, which alter the efficiency of electron transfer, are measured as a change in current. Because of this signaling mechanism, there are a variety of methods to optimize the analytical properties of these sensors, including sensitivity, dynamic range, and binding affinity. Here, I present a general method to tune the analytical figures-of-merit of representative RNA and DNA-based E-AB sensors. First, varying the voltammetric interrogation frequency and rationally engineering the aptamer sequences with different signaling abilities allows for control over the sensor sensitivity, limit of detection (0.01-12 [micro]M), and observed binding affinity (0.22-42 [micro]M). Second, combining the newly engineered aptamer sequences at varying ratios on the biosensor surface allows for control over the sensitivity and linear range of the resulting sensor. These sensor guidelines represent a universal method for the development of electrochemical, aptamer-based biosensors with desired performance attributes.

Keywords: Bioanalytical, Biosensors, Electrochemistry, Electrodes
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Accurate measurements of environmental exposure are central to determining the risk of hazardous substances. National Institute of Environmental Health Sciences (NIEHS) Superfund Research Program (SRP) invests in development of fast, accurate, robust, and advanced technologies that allow for portable real-time, on-site characterization, monitoring, and assessment of contaminant concentration and/or toxicity. This presentation highlights SRP innovations in devices and tools for exposure assessment of organic, inorganic and mixtures of contaminants with a particular focus on passive sampling devices used in contaminated sediments. Featured research will include: recent advances in a universal passive sampling device (PSD) for measuring the time-weighted-average chronic exposure to hundreds of organic chemicals in water; wearable PSDs to measure the bioavailable fraction of PCBs, PAHs, and their metabolites in water, sediment, and soil; extraction techniques utilizing a convenient repository sample collection device for practical shipment, storage, and analysis of aliquots (both environmental and biological samples); in situ sampling devices for measurement of emerging sediment contaminants (fipronil, triclosan, and triclocarban); newly developed polymeric materials for passive sampling during remediation efforts using activated carbon sorbents in the field for PCB impacted sediments; and employing stable isotope labeled of hydrophobic organic compounds can improved the efficiency and accuracy of bioavailability measurements from PSDs. Looking forward, SRP research is advancing detection science to enable rapid assessment of bioavailable fractions of hazardous substances in the environment; and development of innovative detection technologies for combined exposures. Lessons learned from field application of these new tools will be discussed, including the technical, policy, and other practical issues that must be addressed for new technologies.

Keywords: Air, Biotechnology, Environmental/Waste/Sludge, Soil
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Oil and Grease analysis is a simple measurement that can give an indication of water quality indicating a variety of possible pollutants. The determinative step is often gravimetric, also simple to use and balances are easily obtainable. This method is used in various forms around the world and is written into a variety of regulatory and consensus standards. For example, oil and grease is a method defined parameter and used quantitatively to assess compliance of a waste stream. But for evaluation of a waste site or landfill it may be used as a screening method and further analyses initiated to more specifically determine the amount and type of contamination.

In the US, oil and grease measurement is typically used as a permit parameter in the National Pollutant Discharge Elimination System (NPDES). As an example, more than 15,000 establishments in the state of Alabama are required to control oil and grease discharge to a permit value. Laboratories performing this analysis are generally accredited and subject to audits from state assessors. In addition, they have requirements from clients that constrain the number and quality of samples.

The intent of this paper is to address some of the more common audit findings or individual concerns using statements taken directly from the method itself or from official releases made by EPA. In addition, a special focus will be made with regards to addressing both user and auditor concerns regarding the use of automated Solid Phase Extraction (SPE) for this analysis. Implementation of method requirements, also consistent with client needs will be discussed.

Keywords: Environmental Analysis, Hydrocarbons, Solid Phase Extraction

Application Code: Environmental

Methodology Code: Sampling and Sample Preparation
Air quality monitoring and investigation applications can be challenging due to the chemical complexity of the air. Since traditional gas chromatography-mass spectrometry (GC-MS) requires daily calibration checks, the number of compounds that can be quantitatively determined is typically limited to approximately 100 compounds. However, most air samples contain hundreds of chemical compounds making it impractical to calibrate for all of them.

Coupling of gas chromatography (GC) with a novel spectroscopic analyzer (MAX™) combines the compound separation of the GC with the qualitative and quantitative capabilities of optical spectroscopy. Unlike mass spectrometry detection, the MAX™ technology calibration is constant so a one-time characterization of the compound is the only requirement. This allows for the quantitative determination of all compounds in every sample without individual compound calibration beyond the initial characterization.

An initial demonstration was performed for several hundred compounds typical of most indoor air environments. Subsequently, representative air samples were collected and analyzed via this method to determine the feasibility. Comparison of these results with traditional GC-MS demonstrates that this technology can provide similar compound identification with better analytical accuracy.

This new combination of GC compound separation with MAX™ detection represents a significant improvement in the ability to separate and quantify complex mixtures such as indoor air with reduced calibration requirements while maintaining low detection limits.

Keywords: Air, Data Analysis, Gas Chromatography, Volatile Organic Compounds
Application Code: Industrial Hygiene
Methodology Code: Gas Chromatography
As helium continues to become more expensive and its availability remains not always consistent, many laboratories are searching for adopting more cost effective carrier gas options.

This study demonstrates the effectiveness of a purge-and-trap preparation system's automatic nitrogen purge feature in conjunction with an innovative gas chromatography split splitless injector capable of using two different gases for its operations.

While helium is used as carrier gas, maintaining the same existing performance normally obtained another less expensive and more common gas as nitrogen is used for the injector split and the purge-and-trap purge phase.

High quality data will be presented for the most common USEPA VOC Methodologies obtained with such instrumentation without time-consuming method revalidation and while also reducing helium consumption. Data on calibration and method detection limit (MDL) samples prepared, analyzed and quantitated according to US EPA methods will be presented and the total potential helium savings of such a system for continues operations will be discussed as well.

Keywords: Gas Chromatography/Mass Spectrometry, Purge and Trap, Volatile Organic Compounds, Water

Application Code: Environmental

Methodology Code: Sampling and Sample Preparation
US EPA method 625 is a general semivolatile method for wastewater analysis applied to a large suite of target analytes. Although method 625 was developed a number of years ago, through the EPA Office of Water, Office of Science and Technology, the method has been updated several times. As new technology is developed either for the determinative measurement or, earlier in the analysis process, for the sample preparation, data must be collected to demonstrate that the new technology is compliant and reproducible. Two small round-robin studies using multiple vendor products and solid phase extraction (SPE) materials in a variety of laboratories have been run to demonstrate the compliance of SPE with method requirements. The first study relied on the quality control in the existing method to catch when the equipment or sorbent did not work properly. In the second study, the choice of surrogates was enlarged to ensure that errors not caught with the criteria in the older version of 625 would be identified in this version.

This paper will discuss the performance of SPE in general for method 625 and the specific performance of a disk used with a single pass of acidified water through it rather than a pass with basified water and a second pass with the same water, now acidified, which is typical for liquid-liquid extraction. Recoveries of a large suite of compounds from a variety of matrices and laboratories will be examined and the effect of surrogates will be considered. The results from the first round robin demonstrated recoveries from 70-130% of most all the acid/base/neutral/pesticides chosen for the study using a multi-mode disk adsorbent. These results will be compared to results from the second study and comparisons of large and small volumes of sample, different surrogate mixes and interlaboratory performance will be discussed.

Keywords: Environmental/Water, GC-MS, Sample Preparation, Solid Phase Extraction
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Liquid chromatography - mass spectrometry (LC-MS) is a powerful technique for analysis of the metabolome due to its sensitivity, selectivity, and versatility. However, metabolomic studies are still challenging due to the vast size and diversity of the metabolome. Polar metabolites are not well retained on a reversed phase column, often requiring separate analysis from less polar metabolites. This can be avoided by derivatizing metabolites with a hydrophobic tag, increasing retention as well as electrospray ionization efficiency. [sup]13[/sup]C-labeled internal standards are frequently added to improve quantification, which is a daunting task for a large sample size of metabolites. Derivatization simplifies this task, as [sup]13[/sup]C-labeled derivatizing agents can be used to label [sup]12[/sup]C standards and generate a stable isotope-labeled internal standard for every analyte.

We have used benzoyl chloride and aniline as two such derivatizing agents. [sup]13[/sup]C-labeled versions of both are commercially available, further simplifying the production of internal standards. Benzoyl chloride reacts with primary and secondary amines, phenols, and thiols, while aniline reacts with carbonyls, carboxyls, and phosphoryls. Benzoyl chloride increases sensitivity 10- to 1000-fold, and has been demonstrated in a directed assay for 66 metabolites, including key components of tryptophan and phenylalanine metabolism. Aniline has been used to target 18 metabolites to date. These complementary techniques are capable of targeting a significant portion of the metabolome. Here the advantages of benzoyl chloride and aniline derivatization are demonstrated in metabolomic studies in a variety of biologically relevant samples, including cerebrospinal fluid and [i]Drosophila[/i] tissue homogenate.

Keywords: Bioanalytical, Derivatization, Liquid Chromatography/Mass Spectroscopy, Metabolomics
Application Code: Genomics, Proteomics and Other 'Omic
Methodology Code: Liquid Chromatography/Mass Spectrometry
Sugar and humectants are additives in tobacco products that can impart positive sensory attributes, and could serve as precursors for potential addictive and harmful chemicals in smoke. We developed and validated an isotope dilution liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) method with high sensitivity, throughput, selectivity and accuracy for the simultaneous determination of sugars (fructose, glucose, mannose, sucrose, and maltose), alditols (xylitol, sorbitol, and myo-inositol), and humectants (glycerol, propylene glycol, and triethylene glycol) in tobacco products. Determination of these tobacco components is important to aid in the ability to characterize tobacco ingredients and their potential effects on acceptability, smoke chemistry and toxicology, and to distinguish tobacco product categories. The developed method provides recovery levels for the targeted analytes ranging from 90% to 113%, limits of detection ranging from 0.0002 to 0.0045 µg/mL and high precision with coefficients of variation ranging from 1.4 to 14%. Calibration curves for all analytes were linear with linearity R² values greater than 0.999. Each LC-MS/MS run takes 15 min. Different tobacco products were characterized using the developed method. Differences in total sugar and total alcohol were observed: Cigarette total sugar range 3.37 - 9.09% and total alcohol range 1.05 - 4.59%; little cigar total sugar range 0.06 – 1.11% and total alcohol range 1.10 - 3.34%; cigarillo total sugar range 0.07 – 15.12% and total alcohol range 0.76 -10.69%. The calculated total sugar levels could unambiguously distinguish little cigars from cigarettes examined. The results obtained from this method could be further used to study the correlations between sugar or humectant levels and tobacco smoke products, and to investigate how different tobacco compounds would affect the levels of sugar or humectant related tobacco smoke products.

Keywords: Liquid Chromatography/Mass Spectroscopy
Application Code: Consumer Products
Methodology Code: Liquid Chromatography/Mass Spectrometry
The ability to detect and identify metabolites and lipids makes metabolomics a valuable tool for biomarker research. Examination of endogenous metabolites and lipids can reveal the fine detailed information regarding active metabolic pathways and biological process occurring in living cells.

Samples undergoing mass spectrometric analysis should be prepared in an unbiased and reproducible manner that maintains the integrity of intracellular compounds. Cellular metabolomic studies are challenging because universal workflows for cell quenching, cell separation from medium or ideal cell washing solutions, and metabolite/lipid extraction methods do not exist. This work includes optimization of a sample preparation workflow for LC-MS cellular metabolomics studies of primary and established suspension T cells to identify untargeted metabolites and lipids involved in Type 1 Diabetes.

Type 1 Diabetes (T1D) results from the auto-immune destruction of insulin-producing pancreatic beta cells by white blood cells or T-lymphocytes. T cells, which normally direct the immune system to counteract infections, are defective and play a large pathogenic role in the mediation of diabetogenesis. This work monitors the metabolic profile of T cells in a normal and high glucose environment to identify biomarkers of immune dysregulation using commercially available Jurkat T-lymphocyte cells as a model system. The metabolic profile of CD4+ t cells from Type 1 Diabetic patients and 1st-degree relatives (controls) are compared to the in-vitro model system to determine putative biomarkers.

This work is funded by SECIM (NIH Grant #U24 DK097209) and the JDRF Research Grant (#17-2012595).

Abstract Title: LC-MS Cellular Metabolomics Methodology for Type 1 Diabetes Using CD4+ T cells

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Keywords: Liquid Chromatography/Mass Spectroscopy, Method Development, Metabolomics, Metabonomics

Application Code: Genomics, Proteomics and Other 'Omic

Methodology Code: Liquid Chromatography/Mass Spectrometry
Electron ionization can significantly benefit LC-MS through the provision of automated library identification and extensive fragment information. Thus, bringing back EI to LC-MS is highly valuable, if a reliable and robust EI interface can be developed.

We developed a novel EI-LC-MS approach, based on interfacing LC and MS with supersonic molecular beams (SMB) and sample ionization with electrons as vibrationally cold compounds in the SMB.

The output of the LC is sprayed via a pneumatic spray at about 1.5 Bar, followed by fast, thermal vaporization of the sample compounds. The vaporized sample passes a 20 cm 0.25 mm ID fused silica flow restrictor capillary into a supersonic nozzle and expands into the vacuum system from a 300 µm supersonic nozzle at about 0.2 Bar pressure to suppress cluster formation while obtaining efficient vibrational cooling for the electron ionization of cold molecules in the SMB.

EI-LC-MS with SMB provides several important benefits including:

A. Library based identification with names and structures.
B. Enhanced molecular ions and MS information with EI of cold molecules in SMB.
C. No ion suppression or enhancement effects are exhibited.
D. Uniform semi-quantitative ionization yields is provided for the provision of chemical reaction yields and improved impurities analysis.
E. Broad range of compounds including non-polars are amenable for analysis.
F. Fast LC-MS is enabled via no ion suppression and deconvolution software (AMDIS) and fast flow injection MS-MS can replace lengthy LC-MS.
G. GC-MS and EI-LC-MS can be integrated into a one system with method based switching.

Keywords: Instrumentation, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry, Organic Mass Spectrometry
LC/MS - 'Omics and Others

Techniques to Achieve Higher LCMS Sensitivity

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LCMS instruments get more sensitive with each new product introduction. Every year, new instruments that have Femtogram and Attogram sensitivity are becoming readily available. Parts per trillion and even quadrillion are becoming routine. Even though LCMS instruments are now capable of such performance, actually achieving detection of such low level amounts reliably is getting more difficult. Contaminants that you never knew about are getting in the way of good quantitation. And getting reproducible results at such low levels leaves a lot to be desired.

This presentation will illustrate some of the ways low level samples are lost, explore what goes bad in the mobile phase, and how to get back all the sensitivity you want. A major emphasis for this presentation is how samples get contaminated, by what chemicals, and how to prevent this from happening. Also to be discussed are some of the artifacts and impurities that are becoming more common as instruments become more sensitive, and we will explore some practical techniques for sensitivity enhancement.

Keywords: Contamination, Liquid Chromatography, Liquid Chromatography/Mass Spectroscopy, Ultratrace Analysis
Application Code: General Interest
Methodology Code: Liquid Chromatography/Mass Spectrometry
Silver nanoparticles (AgNPs) have been incorporated into a broad variety of consumer products because of their bactericidal properties. Because of the unique nature of nanoparticles, silver nanoparticles have the ability to deliver silver ions to targeted toxins more effectively than silver in its salt form (Ag+). The release of Ag+ from AgNPs can be directly attributed to the nanoparticle size and surface modifications. Methods are in demand to discriminate AgNPs with a variety of surface modifications from Ag+ and also separate AgNPs by size. Although a variety of peer reviewed silver/nanoparticle methods have been established, there are still gaps with regard to the ability to separate small silver nanoparticles (>20nm) from Ag+.

Recently, chromatography with inductively coupled plasma mass spectrometric (ICP-MS) detection has been explored as a means for nanoparticle size separations and differential detection of AgNPs from Ag+. Traditional techniques such as field flow fractionation and capillary electrophoresis have demonstrated the capability to separate AgNPs by size with Ag+ eluting during the exclusion time. However, these techniques require relatively clean matrices and the very smallest nanoparticles may coelute with silver complexes, neutral species or Ag+. High performance liquid chromatography (HPLC) methods are becoming more common as a tool to do AgNP size separations and differential detection of Ag+ from AgNPs as shown by Bettmer, et al. and Hanley, et al. HPLC in tandem with ICP-MS offers element specific detection and the flexibility of being able to separate the smallest of nanoparticles from Ag+.

The proposed study demonstrates how chromatography can be used in tandem with diode array/ICP-MS detection to separate silver nanoparticles functionalized with a variety of agents by size while isolating Ag+. The goal is to provide a separation where the AgNP surface modification is conserved and does not control the ability of the AgNP to elute.

Keywords: Chromatography, ICP-MS, Nanotechnology, UV-VIS Absorbance/Luminescence
Application Code: Nanotechnology
Methodology Code: Liquid Chromatography/Mass Spectrometry
Simultaneous Quantification of Inorganic and Organic Mercury Species in Drinking Water at Single-Digit Picogram Levels by Direct Speciated Isotope Dilution (D-SID) IC-ICP-MS

Analysis of mercury species in drinking water is being reduced to parts-per-trillion level (pg/g of water) by regulatory bodies in the US and this trend is also observed worldwide. Naturally occurring mercury has been widely distributed by natural processes such as volcanic activity. The use of mercury in industrial processes significantly increased following the industrial revolution of the 19th century. Today coal fired power plants and crude oil are the largest sources of environmental mercury species and continue. The solubility of mercury compounds in water varies according to their molecular form. The elemental mercury is insoluble, mercury (II) chloride is readily soluble, mercury (I) chloride is much less soluble and mercury sulfide has a very low solubility. Methylation of inorganic mercury is an important process in water and occurs in both fresh water and seawater. The environmental levels of methylmercury depend on the balance between bacterial methylation and demethylation. New methods are required for the preconcentration, separation and quantitation of the mercury species such as inorganic mercury ions (Hg+ and Hg2+), methylmercury (CH3Hg+), ethylmercury (C2H5Hg+) and others. In the present study, a new method of ion chromatography ICP-MS using Direct Speciated Isotope Dilution method has been developed and is described that has achieved quantification of mercury species in one batch using IC-ICP-MS with quantification levels at the pg/g level in drinking water.

Keywords: Environmental/Water, ICP-MS, Ion Chromatography, Water
Application Code: Food Contaminants
Methodology Code: Liquid Chromatography/Mass Spectrometry
It is well known that the elution strength of the solvent of your sample diluent can cause significant effects on peak shape in gradient elution chromatography. Depending upon the volume of the injection, these strong solvents can even cause early eluting analytes to break through the column, resulting in no retention. There are several reasons why it might be desirable, or even necessary to inject samples in strong eluting solvents. One of these cases would be for samples that are not soluble, or are sparingly soluble in the initial mobile phase, and need larger concentrations of organic solvent. Another example would be in a case where a solid phase sample cleanup step requires strong organic solvent for analyte recovery. Usually in this latter case, the sample is blown down to concentrate the sample, and then reconstituted in weaker eluting solvent. However, this step is time consuming and it would be desirable to be able to directly inject the sample without need for the blowdown step. In this presentation we will show a sample injection strategy that allows for the injection of relatively large volumes of strong elution solvent, while significantly reducing sample break through and peak shape distortions of early eluting peaks. The ultimate goal of this work is to be able to directly inject protein precipitated and other pretreated samples directly, in high enough volumes for adequate sensitivity, while still maintaining good chromatographic performance.

Keywords: HPLC, Liquid Chromatography, Liquid Chromatography/Mass Spectroscopy, Sample Introduction
Application Code: Clinical/Toxicology
Methodology Code: Liquid Chromatography/Mass Spectrometry
Tricresyl phosphate (TCP) is an organophosphate used as an additive in a variety of applications including industrial lubricants, hydraulic fluids and gasoline. Commercial TCP consists of a mixture of ten positional cresyl isomers. The ortho isomers are considered to be the most harmful, exhibiting neurotoxic properties. Human exposure to tri-ortho-cresyl phosphate (ToCP) can cause peripheral nerve damage and degeneration of the spinal cord. The largest reported ToCP exposure occurred in the United States during the 1930s from the consumption of ToCP-adulterated Jamaica Ginger. Approximately 50,000 people experienced paralysis often referred to as the “Jake leg”. The neurotoxic effects of ToCP arise from the liver-activated metabolite cresyl saligenin phosphate (CBDP). Similar to other organophosphates, CBDP inhibits esterase enzymes including butyrylcholinesterase (BChE). CBDP forms a covalent bond with BChE at the serine 198 position. Following BChE adduction, CBDP undergoes hydrolysis to form two aged adducts. Ortho-cresyl phosphoserine BChE (oCP-BChE) is formed immediately and a second aging step produces phosphoserine-BChE (P-BChE). Currently, no laboratory assay is available to quantitate human exposure to ToCP. In this work, an immunomagnetic-UHPLC-MS/MS method was developed to quantitatively measure the long-term biomarkers oCP-BChE and P-BChE in human serum. The reportable range of adduct concentration was from the lowest reportable limit 2.00 ng/mL to 150. ng/mL, which is consistent with the sensitivity of methods used to detect organophosphate nerve agent adducts.
Mycotoxins are ubiquitous food contaminants produced by filamentous fungi. Human exposure to mycotoxins occurs via contaminated food intake, inhalation and/or dermal contact. Studying mycotoxin contamination is of utmost importance today because of their diverse effects on human health. The objective of this work was to develop a sensitive and reliable multi-mycotoxin assay allowing simultaneous detection and quantification of common toxicologically important mycotoxins and their metabolites: ochratoxin A, aflatoxins B1, B2, G1 and G2, zearalenone, 7[alpha]-hydroxy-zearalenol, 7[beta]-hydroxy-zearalenol, zearalanone, 7[alpha]-hydroxy-zearalanol, 7[beta]-hydroxy-zearalanol, fumonisins B1 and B2, T-2 toxin, HT-2 toxin, deoxynivalenol, nivalenol, 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol, fusarenon X, and beauvericin in human blood samples. The proposed exposure-monitoring method uses liquid chromatography - mass spectrometry (LC-MS) in combination with a universal sample preparation technique that requires low sample volume and has high sensitivity, speed, and accuracy. Optimization of LC-MS method included detailed comparison of sample preparation techniques (solvent precipitation, solid-phase extraction, solid-phase microextraction and liquid-liquid extraction), sample pH, chromatography, and LC-MS instrumentation (Orbitrap and triple quadrupole). Solvent precipitation was not found suitable for plasma samples for this application. Liquid-liquid extraction and solid phase microextraction did not provide high recovery for polar mycotoxins and fumonisins. The best result was provided by hydrophilic-lipophilic-balanced solid-phase extraction which provided balanced coverage of both hydrophobic and polar mycotoxins. The optimized LC method relied on pentafluorophenyl column with water/methanol/acetic acid mobile phase for best sensitivity. The optimized method was validated according to regulatory guidelines and is suitable for mycotoxin exposure studies.

Keywords: Bioanalytical, Liquid Chromatography/Mass Spectroscopy, Sample Preparation, Toxicology
Application Code: Clinical/Toxicology
Methodology Code: Liquid Chromatography/Mass Spectrometry
This presentation provides attendees with a complete overview of the medical cannabis industry, from “seed to store”. Medical marijuana (MMJ) refers to the use of cannabis and its corresponding cannabinoids, as a therapy to treat diseases and/or alleviate symptoms. This industry is projected to be an $8B industry by 2018. With this growth has come an explosion in medical marijuana testing labs, which perform testing that spans across many market segments, including medical, food safety, environmental, microbiological and QA/QC to ensure that patients receive safe medicines free from pesticides, contaminants, residual solvents and microorganisms.

This presentation will begin with a visual tour of grow operations and cannabinoid extractions. Current forms of medical cannabis products will be presented along with considerations for patient safety. The focus of the presentation will be an in-depth look at lab testing, highlighting the analytical instrumentation (including LC, LC-MS and GC-MS) used for profiling and potency testing as well as other typical lab services, such as pesticides/fungicides, residual solvents and microorganisms. This review will include an overview of sample prep and cannabinoid extraction techniques.

While THC is the most abundant cannabinoid (known as a mild analgesic with antioxidant activity), there are over 70 different cannabinoids in marijuana, with 8 commonly tested. Knowledge of both the cannabinoid profile and potency is essential in allowing the patient to choose the correct medicine for their needs, as well as determine dosing. The known therapeutic effects of these phytocannabinoids will be explored, with a look into future opportunities for personalized medicine therapies. Lastly, this presentation will conclude with opportunities for future cannabis laboratory testing, including marijuana strain typing, standards development and certification.

Keywords: Clinical/Toxicology, Food Safety, Pesticides, Sample Preparation
Application Code: Biomedical
Methodology Code: Liquid Chromatography/Mass Spectrometry
This presentation will provide attendees with knowledge on tobacco exposure assessment via quantification of urinary nicotine, six of its metabolites (trans-3'-hydroxycotinine, cotinine, cotinine-N-oxide, nicotine-N-oxide, nornicotine, and norcotinine), and two minor tobacco alkaloids, anabasine and anatabine. Nicotine metabolic profiles are paramount in determining population risk assessments, exposure, and user trends- from traditional cigarettes to newly emerging tobacco products.

Sample preparation utilizes a customized robotic automation system that includes sample aliquoting, enzymatic hydrolysis, incubation, acetone precipitation, centrifugation, and evaporation. The accuracy and precision of the system allows for reliable plate preparation overnight; current method configuration prepares four 96-well plates each day, compared to the manual method, which takes two days for a single plate.

Samples are quantified using isotope-dilution LC-MS/MS with an analytical run time less than 8.5 minutes. Analyte MS de-tuning allowed for the quantification of all compounds, despite significant concentration variations across their reference ranges in urine. Automated integration software was used to reduce data processing time by hours per run based on customized QA rule settings and optimized peak picking parameters.

The validated method was applied to ~100 smoker samples to assess nicotine exposure and individual metabolism characteristics. The correlation of individual analytes to the total nicotine exposure indicated that cotinine was the preferred biomarker to measure compared to trans-3'-hydroxycotinine, despite trans-3'-hydroxycotinine concentrations in smoker urine being quite high compared to that of cotinine. Trans-3'-hydroxycotinine-to-cotinine ratio variation persisted (Range= 0.2-7.1), indicating a high degree of variability in CYP-450 2A activity among individuals, yielding both fast metabolizers and slow metabolizers.

Current and future analyses will focus on measurement in samples from national cohort studies in collaboration with Roswell Park Cancer Institute, NIH; FDA, Center for Tobacco Products; and UCSF, Center for Tobacco Control, Research, and Education. Following data release to these studies, a reference level in US smokers will be established.

Disclaimer: This study was funded through an interagency agreement by the U.S. Food and Drug Administration Center for Tobacco Products.
Late Stage Functionalization (LSF) refers to the use of C-H bond activation chemistries for the incorporation of chemical diversity into a final product or advanced intermediate late in a synthetic sequence. LSF technologies offer the exciting possibility of accessing many diverse modifications in a single step from the desired compound of interest, giving discovery chemists an ability to quickly spawn new 'versions' of compounds. This has enabled access to structural diversity and new chemical space without the time consuming necessity of developing entirely new synthetic routes.

The Merck High Throughput Purification (HTP) group has adapted existing purification platforms and work flows to provide the critical chromatographic purification support for chemists utilizing LSF. Due to the possible non-directed nature of the C-H functionalization chemistries, obtaining purified compounds for positive structural identification of the resultant products is a critical step in the work flow. In many instances, a single reaction can produce several positional isomers as well as multiple additions of the desired functional group; in addition, the conversion rates can be low, requiring isolation of low mg quantities of material. These challenging purifications often require application of multiple orthogonal chromatography steps to allow isolation of all relevant products in sufficient quantities and purity for structural identification and subsequent biological assays.

This presentation will highlight the implementation of current HTP work flows toward solving these complex purification problems. This will include application examples utilizing semi-preparative, MS-Directed reversed phase chromatography, as well as both chiral and achiral Supercritical Fluid Chromatography (SFC). Current best practices and lessons learned from this work will also be discussed.

Keywords: Drug Discovery, Isolation/Purification, Prep Chromatography, SFC
Application Code: Drug Discovery
Methodology Code: Liquid Chromatography/Mass Spectrometry
New drug applications require the applicant to provide acceptable analytical methods to assay the drug product for the active pharmaceutical ingredient (API) and related manufacturing impurities and degradants. Analytical methods need to be precise, accurate and suitable for use as quality control methods for use by the applicant and as regulatory methods for use by the Agency. Method robustness can be improved by the use of quality by design principles during development of the method. Commonly encountered reviewer observations regarding validated methods submitted to the U.S. Food and Drug Administration will be discussed.

Keywords: Analysis, Biopharmaceutical, High Throughput Chemical Analysis, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
2D-LC is a powerful separation platform that uses orthogonal separation mechanisms in each dimension to maximize the peak capacity and achieve separation efficiency that cannot be obtained by one-dimensional HPLC. Two case studies were presented to demonstrate the advantage of 2D-LC for the analysis complex biotherapeutics samples.

Antibody drug conjugates (ADCs) are a new class of biotherapeutic agents combing the specific targeting property of antibody and the cytotoxicity of a small molecule drug to achieve targeted delivery of cytotoxic drugs. One important quality attribute of ADCs is the content of the unconjugated toxin which poses toxicity concern. In case study 1, a SEC column coupled with RP HPLC method was utilized to analyze the unconjugated small molecule drug and related impurities in ADC directly without sample preparation. The SEC column in the 1st dimension separates the unconjugated small molecules from the ADC, and the small molecule components were further analyzed by RP HPLC in the 2nd dimension for identification and quantification. This 2D-LC method can be used for both lot release and stability testing of ADCs.

In the case study 2, a mixed mode-RP method coupled with CAD and MS was developed to characterize polysorbate 20 in protein therapeutics. Polysorbate 20 is a surfactant commonly used in protein formulations to prevent protein denaturation and aggregation. It is critical to understand the molecular heterogeneity and stability of polysorbate as it degrades in aqueous solution and loses its surfactant activity. A mixed-mode column that has both anion exchange and reversed-phase properties was used in the 1st dimension to separate the polysorbate esters from the protein, and the esters were then analyzed by a RP-UHPLC column in the 2nd dimension to further characterize the ester subspecies. This 2D-LC method was used to monitor the heterogeneous stability of polysorbate 20 in the stability samples of a protein drug product.
Traditional spectroscopic systems are generally constructed by optical components aligned individually in a space. In addition, optical traps and outside shields are obliged to suppress background noise. To keep positioning accuracy, robust holding mechanisms must be fixed on a concrete foundation, and it makes optical systems larger, heavier, and more expensive.

We focus on optical performance of composite and hybrid material based on the PDMS (polydimethylsiloxane) that has good UV transparency and low reflection on optical contact with glasses. This compact and flexible PDMS optical module can be combined on a tablet terminal as an optical foundation that equips power source, light sources (LCD) and sensor camera. Attached PDMS optical system is monolithic and contains solid optical path, optical components, and light coupling interfaces with the LCD and the camera. Simple PCR tube absorbance-meter (Fig.1) was developed as first model of this Labo-On-Tablet (LOT). Polymeric optical fibers were embedded. PDMS doped with Carbon particle (Shinetsu, KE-COLOR-BL, ABS=72/cm) covered a transparent PDMS optical components to reduce outside background radiation and internal scattering. Furthermore, only reflectance of 0.95% per reflection/scattering was obtained.

Pick-up part of LCD light was newly proposed and developed based on reversed taper coupling scheme. The plastic optical fiber was terminated with slanted angle of 45 degree with no-coated surface. The light from LCD lighting spot was picked up, and guided to the PCR chamber. The transmitted light was collected by the POF on opposite side was bundled and guided to the optical detector (face camera) via prism and lens made of PDMS.

The measurement accuracy was evaluated with Sudan-I water ethanol solution with green LCD lighting. The face-camera captured images of terminated POF surface for 10 times at each concentration of 1 M to 1mM. The lighting spot of fiber was cropped and the pixel intensity was integrated over the spot as a signal. Experimentally obtained signal scattering of ±0.07%std in the 10 times continuous measurement. Since the scattering of ±0.13std was obtained when the PCR tube was ejected and reenter, their reproducibility about 0.05% can be estimated. The absorption sensitivity of 0.001 was also approximated from the calibration curve with single capturing. Thus, sensitivity of 0.0001 will be expected by using multiply capturing and averaging.

Keywords: Biosensors, Lab-on-a-Chip/Microfluidics, Spectroscopy, UV-VIS Absorbance/Luminescence
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Paper-based microfluidic devices are receiving great attention due to the characteristics of low cost, simple fabrication, and spontaneous capillary action. However, slow flow in paper channel limits a device size and a practical use in various analytical applications due to long analysis time. Here, we present a new paper-based flow channel (hollow-paper channel) having empty space and paper within a channel closed by hydrophilic PET films and wax-printed barriers (see the cross-sectional structure in Fig. 1). The center paper layer was fabricated by wax printing and laser cutting. Fig. 1A shows a flow-test device, and travel distance variations of a dye solution as a function of detection time for three different pressures. Even at the pressure of 0 mbar, it exhibited very fast flow (less than 20 s for 10 cm moving), corresponding to a flow rate 6 times higher than that of a paper channel, probably due to the hydrophilicity and a flexible small gap between paper and PET. In addition, flow became faster as increasing a head pressure. With using the hollow-paper channel, we had fabricated colorimetric pH sensors using a bromothymol blue pH indicator. A measured mean gray value was 114, being much higher than the value of 72 in a comparable paper chip (see Fig. 1B). It clearly demonstrates that our new colorimetric sensor with the hollow-paper channel can detect pH very sensitively via uniform color formation and higher sample transfer ratio into the detection zone together with the short assay time.

Keywords: Chemometrics, Lab-on-a-Chip/Microfluidics

Application Code: Bioanalytical

Methodology Code: Microfluidics/Lab-on-a-Chip
Transfusion of erythrocytes (ERYs) can cause complications in patients, especially if the ERYs have been in storage for longer than 14 days. Recent studies have shown that mortality rates are higher following transfusion in comparison to no transfusion. While many reasons exist for these complications, one of particular interest to our group is the decrease in nitric oxide (NO) bioavailability following transfusion. Recently, we reported that ERYs stored in normoglycemic conditions (5-6 mM glucose) released significantly higher levels of ATP (an NO stimulus) when subjected to flow, than those stored in the standard hyperglycemic storage solutions. Here, we report the ERYs ability to revert back to normal function if transferred to normoglycemic conditions, as would be the case in an actual patient transfusion. A 3D-printed device was used to show the flow-induced ATP release from ERYs stored for 1 day in hyperglycemic conditions before (126.8 ± 10.5 nM, n=7) and after (226.1 ± 2.4 nM, n=7) transfer to a normoglycemic buffer. After 12 days of storage, the ATP release was not reversible (113.4 ± 3.7 nM, 135.9 ± 7.4 nM, n=7). Secondly, ERY membrane deformability during storage was examined by a 3D-printed blood filtrometer. Reductions in deformability were observed in hyperglycemic conditions, but were reversible up to day 8; after this point in storage, the ERYs remained permanently rigid. The tools fabricated with 3D-printing technology lasted for the entire timeframe of the study, did not clog due to cell movement, were custom-designed and can be shared with all labs through an electronic dropbox.
A pillar array column (PAC) is a micro column which is fabricated by reactive ion etching of a silicon substrate and has non-porous pillars with a high degree of order in narrow flow channels. Compared to conventional spherical particles randomly packed in an empty column, the order of pillars is systematically designed to provide a higher efficiency due to a smaller van Deemter A term and higher permeability ($K_v$) attributed to higher external porosity. Therefore, PACs are expected to have a higher resolution and are promising candidates for the next generation chromatography columns. However, retention capacity (contributing to resolution) with PACs is low because pillars have a non-porous structure resulting in a low surface area.

In order to achieve the same level of retention factor with PACs as with superficially porous particles which are currently drawing much attention in the market because of the high efficiency, electrochemical anodization was applied to grow porous shell layers of 300 nm (30 nm pores) on the surface of pillars with the diameter of 5 um and spacing (between pillars) of 2.5 um. A specific injection method (on-chip injection) was established, which enables a small amount of injection (a few nL) to minimize an influence of injection volume on a tiny PAC.

Uniformity of the porous PACs were assessed by determining local height equivalent to a theoretical plate (HETP) along the channels, which appeared to be constant. On measuring the retention time as an indicator of the surface area, an increase in the surface area by a factor of about 30 compared to that of non-porous pillars was found as expected. On measuring the efficiency of a PAC with a commercial HPLC instrument, theoretical plate of about 100,000 with a retention factor of 2.2 under an isocratic condition and peak capacity of about 500 under a gradient condition were obtained at an extremely low pressure with a 1 m long PAC, which are ascribed to the increase in surface area.

Keywords: HPLC Columns, Lab-on-a-Chip/Microfluidics
Application Code: High-Throughput Chemical Analysis
Methodology Code: Microfluidics/Lab-on-a-Chip
Microfluidic electrophoretic affinity assays are a popular method for the analysis of complex biological systems. The success of an affinity assay relies on preservation of the non-covalent affinity complex, which can be achieved by decreasing the dissociation rate constant (koff) of the affinity complex during the separation. However, Joule heating produces temperatures as high as ~60 °C which increases koff and therefore complex dissociation. Moreover, the microchip architecture may exacerbate this effect by insulating the channels with the bulk material making it difficult to dissipate localized heating.

To evaluate the heating effects on the sensitivity and performance of affinity assays, a microfluidic device was integrated with temperature controllers and an insulin immunoassay was used as a model assay. Cooling was implemented over the separation domain to preserve the affinity complex and ambient temperature was actively maintained over the sample delivery channel. Separation temperatures of 26, 25, 23, and 21 °C were assessed as well as separation voltages of 3, 4.5 and 6 kV. Optimum conditions were identified by the minimum koff value as determined by kinetic CE. The cooling permitted stable temperatures at higher separation voltages and the assays performed at 6 kV and 21 °C were found to be optimal with 3.4% complex dissociation during the separation. This optimization produced a 10-fold improvement in the LOD to 1 nM. In turn, these contributions will allow for the development of robust affinity assays for the analysis of biological systems.

This work was supported by NIH grant DK080714.

Keywords: Bioanalytical, Electrophoresis, Immunoassay, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
### Novel Microfluidic Instrumentation and Devices

#### Microfabricated Sampling Probes for Minimally-Invasive Neurochemical Monitoring with High Spatial Resolution

Sampling probes, i.e. microdialysis and low-flow push-pull sampling, are essential tools for in vivo monitoring of neurochemicals. However, conventional sampling probes are typically handmade and have several limitations: 1) large in size (probe diameters are over 200 [μm]), resulting in inevitable tissue damage and poor spatial resolution; 2) limited flexibility in their designs; and 3) tedious to make by hand. To overcome these disadvantages, we have used a microfabrication approach to develop miniaturized sampling probes.

Briefly, microfabrication of sampling probes was performed within a class 1000 cleanroom (Lurie Nanofabrication Facility at UMich). The probes were fabricated from 525 [μm] thick N-type 100 mm silicon wafers coated with SiO[2] mask. A series of microchannels were patterned on a wafer by photolithography before the wafer was etched by plasma etching tools, i.e. deep reactive ion etching (DRIE) and XeF[2] etching tools. The wafer was then sealed with poly Si using low pressure chemical vapor deposition. Lithography and DRIE were again used to assign and etch probe shapes and sampling areas with desired dimensions. Additionally, for microdialysis probes, nanoporous anodic aluminum oxide was adapted for use as a nanotemplate for making sampling membranes.

Microfabrication allows us to incorporate smaller sampling areas and three 20 [μm] wide microchannels within a probe, which has overall dimension of less than 100 [μm]. Furthermore, over a hundred probes can be fabricated within a wafer. Preliminary studies have shown suitability for sampling from small brain regions in live rodent brain.

**Keywords:** Bioanalytical, Lab-on-a-Chip/Microfluidics, Neurochemistry, Sampling

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Fluorescence polarization immunoassays (FPIA) have been used as alternative methods to electrophoretic immunoassays, ELISAs, and other quantitative affinity assays. FPIAs are attractive because they require no separation or washing steps. However, multicolor FPIAs have not yet been reported, restricting FPIAs to single analyte applications. This limitation is primarily due to the increase in complexity of the optics required for simultaneous multi-analyte polarization measurements. Here we address this issue by utilizing frequency encoded laser induced fluorescence thus simplifying the optics required for a multi-analyte FPIA.

Frequency encoding the fluorescence signals allows the use of a single PMT to detect multiple emission lines without additional filters. The sample is excited with a 635 nm diode laser pulsed at 73 Hz and a 488 nm laser pulsed at 101 Hz, additional lasers can be added for more fluorophores. The resulting fluorophore emission pulses at the same frequency as the exciting laser and we can isolate the individual signals using Fourier analysis. This allows the use of a two PMT set-up to measure vertically polarized and horizontally polarized light split by a polarizing beam splitter and the separate analytes to be encoded in the signals using frequency. We demonstrated the method with an insulin and glucagon immunoassay, which are important peptides for blood glucose regulation. The LOD for is ~10 nM for insulin and ~50 nM for glucagon, which suffices for monitoring for collections of ~10 islets of Langerhans, the endocrine units of the pancreas.

This work is funded by NIH Grant DK080714.
Enzyme linked Immunosorbent assay (ELISA), usually performed on multi-well plates, is one of the most widely used laboratory diagnosis methods. However, the applications of ELISA in low-resource settings are often limited by long incubation time, consumption of large volumes of precious reagents, and expensive and sophisticated equipment. Herein, we developed a simple, miniaturized PMMA (Poly (methyl methacrylate)) /paper hybrid microfluidic ELISA microplate for low cost and high throughput infectious diseases diagnosis (Figure 1). The novel use of paper inserted in microwells in this hybrid microplate facilitates rapid antigen immobilization, avoiding complicated surface modifications. The top reagent delivery channels and vertical flow-through wells in the middle PMMA layer can simply transfer reagents to multiple wells (7x8 wells), thus avoiding repeated manual pipetting and washing steps in conventional ELISA or the use of costly robots. Unlike traditional microplates, ELISA can be completed within an hour in this microplate. Additionally, results of colorimetric ELISA could be observed by the naked eye. Quantitative analysis was achieved by calculating the brightness of images scanned by a desktop scanner. Using this hybrid microplate, IgG and hepatitis B surface antigen (HBsAg) were analysed. Although no specialized ELISA equipment was used, the limits of detection of 1.56 ng/mL for IgG and 1.60 ng/mL for HBsAg have been achieved, which are comparable to commercial ELISA kits. We envisage that this hybrid microplate can be used to perform enormous bioassays that are currently performed in traditional microplates, in resource-limited settings.

Financial support from NIH and UTEP MRAP is gratefully acknowledged.

Keywords: Immobilization, Immunoassay, Lab-on-a-Chip/Microfluidics, Quantitative
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Sodium deoxycholate (NaDC)/ tris(hydroxymethyl)aminomethane (TRIS)-based hydrogels have been developed as multipurpose drug delivery vehicles. Specifically, we have recently formulated NaDC/TRIS hydrogels for the traditionally difficult application of enantioselective drug release. Such a delivery system could eliminate the need for chiral separation prior to drug loading in many cases. Total release and release rate from this system have been found to be insusceptible to several modifications and additions to the hydrogel. This is a great benefit in that additives to drug delivery systems may be desirable in many applications. For example, a modifier may be added for increased mechanical strength or complementary health benefits. In addition to robust enantioselectivity, benefits of our hydrogel system include low cost, facile synthesis, tunability, high drug loading capability, drug loading concurrent with gelation, and a complete lack of organic solvents. Drug release also seems to be unaffected by gelator concentration, further reducing cost and minimizing required precision and skill for synthesis. Through use of UV-vis absorbance spectroscopy, enantioselective drug release was found to be tunable with hydrogel formulation, drug composition, and drug concentration. Conclusively, our NaDC/TRIS hydrogels can serve as distinctly beneficial, enantioselective drug delivery systems.

We would like to acknowledge funding for this work from NSF CHE – 1307611 and the Louisiana Board of Regents.

**Keywords:** Bioanalytical, Bioinformatics, Biopharmaceutical, Chiral

**Application Code:** Pharmaceutical

**Methodology Code:** UV/VIS
We have evaluated the variation of accuracy for the determination of active pharmaceutical ingredient (API) concentration when the particle size of samples changes. When Raman spectroscopic compositional analysis of powder mixture sample is attempted, the variations in the physical properties of samples such as particle size and packing density have confounding effects on resulting spectral features. Especially, the characteristics of Raman scattering events could sensitively vary depending on particle size although the composition of sample remains unchanged. For the evaluation, binary mixture samples of ambroxol and lactose were prepared with 4 different particle sizes of lactose (under-50, 50-100, 100-150 and over 150[μm]), while the particle size of ambroxol maintained constant (15-30[μm]). The ambroxol concentrations in the samples varied from 9.0 to 15 wt% with increments of 0.5 wt%. Both back-scattering and transmission Raman spectra of the samples were acquired, and the resulting spectra features were examined in relation with the particle size. The shapes of Raman bands were slightly different when the particle size changed. Then, using 4 different datasets prepared using the 4 different particle sizes, partial least squares (PLS) regression was individually performed to determine ambroxol concentrations and the resulting accuracies were compared. The accuracy varied according to the particle size and improved when the particle size was smallest. To probe the origin of the varied accuracy, the propagation of laser and Raman photons in the samples were simulated and variation of spectral feature according to the particle size was investigated using Monte-Carlo method.

Keywords: Analysis, Infrared and Raman, Light Scattering, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Vibrational Spectroscopy
In the production of drug tablets, it is important for pharmaceutical companies to have control of the solid state form of the active pharmaceutical ingredient (API). Process induced transformations can occur to the API during the various production stages. Potential transformations include polymorphic changes, transformation to a hydrate crystalline form, or the formation of amorphous regions. Such changes in the crystalline form of the API can affect the solubility, bioavailability, and stability and thus change the efficacy of the drug tablet. Wet granulation is one processing stage that can cause this type of transformation. During wet granulation an aqueous solution is added to the dry components to form granules. Certain anhydrous crystalline APIs will transform to the hydrate crystalline form when this occurs. One way to slow down or stop this transformation is with the use of polymeric excipients. In this study three model APIs, caffeine, theophylline, and carbamazepine were investigated. The anhydrous API was added to aqueous solutions containing different polymeric excipients and the transformation to the hydrate was monitored using in-line Raman spectroscopy. The collected spectra were correlated to a partial least squares (PLS) regression model to quantify the extent of transformation of the API. The results showed that for each of the APIs different polymeric excipients showed different inhibiting effects. For example, polyacrylic acid inhibited the transformation of caffeine and theophylline but showed little inhibition for the carbamazepine transformation. In addition, there was a pH effect for the polyacrylic acid inhibition indicating the importance of the hydrogen bond donor interaction of the polymer sidechain with the API crystal.
NIR Spectroscopy for Pharmaceutical Solid Analysis: Challenges, Mitigations and Beyond

NIR has become a routine tool in the analysis of solid samples due to its ability to rapidly provide valuable chemical and physical information non-invasively. NIR is a well established spectroscopic tool for process monitoring, process control and real-time release methods in a variety of industries. Even with these advantages application of NIR in the pharmaceutical industry has been limited, in part due to the complexity of robustly extracting a specific response from a complex solid mixture. In this presentation, we will discuss some of these challenges and potential solutions to minimize or eliminate them.

In order to develop an NIR method to address a particular problem, one first has to understand what NIR is interrogating during the analysis. Knowledge of the sampling volume is essential to understand the statistical response of the system and will be considered in this presentation. In addition, the influence of matrix properties, e.g. other components, agglomeration of analytes, sample density, or powder adhesion/cohesion, will also be discussed. As NIR spectra contain both chemical and physical information an understanding of how scattering and absorption is essential when measuring samples. A specific example will be presented that demonstrates the role of powder surface area and particle size distribution on NIR method performance for powder blending. Finally, the effect of environmental factors such as relative humidity will be exemplified.

Abstract Text

Keywords: On-line, Process Analytical Chemistry, Process Control, Spectroscopy
Application Code: Pharmaceutical
Methodology Code: Process Analytical Techniques
Abstract Text

Quality by design (QbD) is a central part of biotherapeutic development, laying the framework for building quality into products through detailed process design and control. Building on the principles of QbD, Process Analytical Technology (PAT) is a critical tool for process understanding and control during manufacture. Implementation of PAT tools provides a means to improve the process and ensure final product quality through real-time monitoring of critical quality attributes (CQAs) allowing for rapid process decisions. In 2004 the FDA published an industry guidance for PAT summarizing the principles for innovative pharmaceutical development. Evolving from the FDA Guidance numerous PAT applications have been studied for the manufacture of both small and large molecule pharmaceuticals. The majority of initial PAT applications monitored process steps through spectroscopic sensors such as NIR, Raman, FTIR or UV-Vis. These techniques function very well for the monitoring of small molecule functional groups or chemical reactions however they lack the specificity and resolution for complex samples such as biotherapeutic proteins.

This work demonstrates the application of the online ultra-performance liquid chromatography system for biotherapeutic development. Successful demonstration of both at-line and on-line sampling followed the separation method of choice. Applications of reversed phase UPLC for titer (RP-UPLC) and purity analysis by ultra performance size exclusion (UP-SEC) or ion exchange chromatography (IEX) will be presented. Cases studies for application of these chromatography techniques for online monitoring of upstream, downstream, forced degradation, and clinical compatibility studies will be reviewed.

Keywords: Biopharmaceutical, On-line, Pharmaceutical, Process Analytical Chemistry
Application Code: Pharmaceutical
Methodology Code: Process Analytical Techniques
A novel electrochemical method has been developed to detect and quantify the elemental impurities, cadmium (II) and lead (II), in pharmaceutical matrices. The approach, involving the use of anodic stripping voltammetry (ASV) on an off-the-shelf, unmodified glassy carbon electrode (GCE), was performed in both aqueous and in dimethyl sulfoxide solutions, without acid digestion or dry ashing to remove organic matrices. Limits of detection in the parts per billion range were obtained for both heavy metals - in the presence and absence of pharmaceutical matrices. The strong reproducibility and stability of the sensing platform, as well as obviation of sample pretreatment show the promise of utilizing ASV as a sensitive, robust, and inexpensive alternative to inductively-coupled-plasma (ICP)-based approaches for the analysis of elemental impurities in pharmaceutical matrices.
Astronauts have available pharmaceutical drugs to overcome the deleterious effects of weightlessness, sickness and injuries. Unfortunately, recent studies have shown that some of the drugs currently used may degrade more rapidly in space, losing their potency well before their expiration dates. To complicate matters, the degradation products of some drugs can be toxic, such as p-aminophenol formed from acetaminophen (Tylenol®), which can cause liver damage. Consequently there is a need for a space-worthy analyzer that can determine if a drug is safe at the time of use, as well as to monitor and understand space-induced degradation, so that drug types, formulations, and packaging can be improved. To address this need we have been investigating the ability of Raman spectroscopy to monitor and quantify drug degradation and determine if a drug is suitable for use based on the presence of 90% or more of the original API concentration. Raman spectroscopy measures the vibrational modes of molecules, which allows identification of virtually any substance, including drugs. Furthermore, it is a “point-and-shoot” technology, in that the sample is simply placed at the laser focal point to make a measurement, typically in 1 minute or less. Here we present preliminary data by measuring a number of drugs, such as acetaminophen, during forced degradation reactions using a compact, low mass, Raman spectrometer.

Keywords: Analysis, Drugs, Portable Instruments, Raman
Application Code: Pharmaceutical
Methodology Code: Vibrational Spectroscopy
The correct identification of raw materials delivered to any manufacturing facility is an important process, none more so than in the pharmaceutical industry where it is a regulatory requirement. For many of the high value pharmaceutical markets 100% verification of incoming goods is mandatory, which puts a significant burden on analytical resources. The need to maintain sterility during the verification process is a further concern for those with aseptic manufacturing or where product cannot be terminally-sterilized as the incumbent methods all require material to be sampled from the containers.

Spectroscopic techniques are increasingly popular for identifying materials due to their speed, convenience and accuracy. It has emerged that the most versatile technique is Raman spectroscopy due to its high chemical specificity and simple operation. A significant limitation of spectroscopic techniques for verification is that the packaging is often not transparent, which precludes the use of NIR, FT-IR and conventional Raman. This means that the samples need to be measured in a sampling booth where the containers can be opened in safety then sampled, resealed and the booth cleaned. This can be extremely expensive, time-consuming and exposes the material to possible bacterial contamination. This report focuses on the application of Spatially Offset Raman Spectroscopy (SORS) to accurate, robust ID through the packaging supplied to the end user. SORS allows high quality Raman spectra to be measured through opaque containers such as sacks, bottles, tubs and bulk containers made out of paper, plastic, glass or multiple layers. We present data on the spectral quality and reproducibility of SORS measurements on common pharmaceutical incoming goods.

Keywords: Biopharmaceutical, Pharmaceutical, Raman, Vibrational Spectroscopy
Application Code: Pharmaceutical
Methodology Code: Vibrational Spectroscopy
The efficacy of many active pharmaceutical ingredients (API) is dependent on either the crystallographic form or even the degree of crystallinity. Indeed, the bioavailability of many APIs is very poor in the crystalline state and must be administered to the patient in amorphous form.

Raman spectroscopy is well-known to be sensitive to polymorphic form, the structural re-organisation of the crystal structure into another stable or meta-stable form. Conventional Raman surface imaging methods for pharmaceutical tablets can be effective at ‘mapping’ the distribution of different polymorphic forms, however, they are limited to one plane within the tablet and the preparation of that flat surface could lead to transformation of the form.

Traditional methods for quantifying amorphicity are powder XRD and solid state NMR (ssNMR). XRD suffers from inherently poor sensitivity for materials with poor or no crystalline structure and may involve sample preparation that could lead to phase transformation. ssNMR is not a routine technique and doesn’t lend itself to supporting production-scale manufacturing and requires high capital expenditure.

Transmission Raman spectroscopy (TRS) offers the potential to rapidly quantify both the proportions of polymorphic form or ratio of crystalline to amorphous API in tablets and capsules with no sample preparation. Crucially, TRS is a true volumetric method and does not suffer from the limitation of conventional Raman imaging. We present data on the technique’s ability to quantify polymorphs and also residual crystalline API in a formulation of amorphous API with sensitivity >10x that of XRD.

Keywords: Raman
Application Code: Pharmaceutical
Methodology Code: Vibrational Spectroscopy
Abstract Text

Quality control of formulated protein drugs presents unique analytical challenges compared to small molecule pharmaceuticals. Proteins are complex macromolecules with secondary and tertiary structures that may easily be perturbed or unfolded, potentially resulting in loss of therapeutic properties. Deep ultraviolet Resonance Raman (DUVRR) spectroscopy is distinctively advantageous for quality assessment of formulated proteins because it is sensitive to changes in protein secondary structure, and is capable of analyzing proteins at concentrations of approximately 1 mg/mL, lower than the typical concentrations of formulated products. The deep ultraviolet resonance Raman enhancement from proteins (approximately 10^4) allowed for analysis in the presence of excipients in aqueous media, which exhibit relatively weaker Raman signals. These attributes have allowed us to analyze the secondary structure of formulated monoclonal antibodies (mAbs) with minimal sample preparation. DUVRR spectroscopy was applied for the quality assessment of formulated Rituxan and Avastin. The formulated products were degraded with chemical, heat and mechanical stress and the degradation products were subjected to DUVRR spectroscopic analysis. Chemical stresses included acidification and addition of surfactant to the formulated product. Changes were observed in the DUVRR spectra with each level of stress, and multivariate statistical models were applied to identify trends within the data. The results indicate that the fully developed method may be applicable as a rapid, sensitive and specific tool to detect degradation of formulated mAb pharmaceuticals.

This research is funded by the CDER Critical Path Initiative.
Liquid chromatography under limiting conditions of adsorption, LC LCD is a technique, which allows separation of macromolecules of distinct chemical composition or physical architecture independently of their molar mass. LC LCD combines exclusion and adsorption retention mechanisms of macromolecules in the column, which is packed by porous particles. Pores are fully accessible to low-molecular substances but macromolecules are pore-excluded. As a consequence, the low-molecular substances elute slowly while the macromolecules elute rapidly. A zone of low-molecular substance, which promotes adsorption of macromolecules, is injected into the column in front of sample. It acts as an impermeable, slowly eluting barrier that decelerates elution of the adsorbing polymer species, while the non-adsorbed macromolecules elute without any delay. In this way, macromolecules that exhibit different adsorptivity can be rapidly, selectively, and efficiently discriminated. The LC LCD method is experimentally well feasible because the results little depend on eluent composition and temperature. It produces narrow peaks of polymers eluted behind barrier and its sample recovery is rather high. LC LCD enables discrimination and identification of minor admixtures present in a polymer matrix, even below 1%. Above merits of LC LCD allow its utilization in two-dimensional polymer liquid chromatography. Important condition for a successful application of LC LCD is adequate solubility of macromolecules in solvents of different polarities. To demonstrate how the latter limitation can be overcome, the low solubility polymers poly(ethylene terephthalate) and poly(butylene terephthalate) with quite similar chemical structure were mutually separated. Above polymers were also easily separated from the aliphatic biodegradable polyesters, polylactides and poly(butylene adipate). The constituents of a model four-component polymer blend were base-line discriminated, as well.
Polypropylene (PP) is a thermoplastic polyolefin widely used in diverse applications for its versatile properties. PP pellets require several mechanical property tests during production before the product is approved and released to the market. There is a great deal of interest in developing and implementing new analytical methodologies capable of providing the necessary information in less time, and hence, reducing cycle time during manufacturing. Raman spectroscopy is an attractive analytical technique for polymer materials because it is reproducible, requires no sample preparation, and provides typical spectra with sharp and well-resolved bands with structural chemical information. Polypropylene (PP) pellets obtained from an industrial plant process were used to develop a nondestructive and faster methodology to quantify mechanical properties using Raman spectroscopy. Raman spectra were recorded from 219 samples of the homopolymer PP, random ethylene-propylene copolymer, and impact ethylene-propylene copolymer. Multivariate calibration models were developed by relating the changes in the Raman spectra to mechanical properties determined by ASTM tests. The spectra were randomly divided into calibration (n=167) and validation (n=52) subsets. Calibration models were developed to predict Young’s traction modulus, tensile strength at yield, elongation at yield on traction, and flexural modulus (1% secant). The best multivariate calibration models were obtained when a combination of genetic algorithms (GA) and partial least squares (PLS) was used on Raman spectral data with relative errors of less than 9%.

Keywords: Chemometrics, Raman
Application Code: Polymers and Plastics
Methodology Code: Chemometrics
Identification and quantification of key chemical species exocytosed by single cells is important for correlating cellular stimuli-responses to normal and diseased phenotypes. Measuring these chemicals is analytical challenging because some species, e.g. ATP, lack innate optical or electrochemical activity. To address this challenge, we are developing a biosensor platform in which ligand-gated ion channels (LGICs) are reconstituted into black lipid membranes (BLMs) that are suspended across apertures in glass micropipettes. We have previously demonstrated that a conductance-based measurement for quantifying ion channel activity can achieve 30 millisecond temporal resolution. We now present work towards realizing submillisecond resolution, using an ATP-sensitive, K+-channel, Kir6.2 as our model LGIC. Using quartz pipettes with 3-10 µm apertures and capacitance cancellation, BLM capacitance charging decayed in <100 µs for potential steps <10 mV, and steady-state current could be sampled 100-200 µs afterward. With three different potential steps, membrane conductance could be sampled every 0.9 ms, making this approach reasonable for monitoring chemical release from single cells at biologically relevant timescales. To demonstrate sensor applicability, we modulate Kir6.2 activity with the antagonist, ATP, and agonist, oleyl CoA, in a system which rapidly exchanges solution across the BLM.

Funding was provided by NIH RO1 GM095763 and NIH R01 EB007047.
Meaningful characterization of molecular processes occurring within living systems requires monitoring and/or quantitation of biomarkers with high accuracy and precision. Levels of many markers present in qualitatively different states may vary so little that their reliable quantification becomes a nontrivial challenge. The task requires availability of sensing tools capable to operate with precisely defined sensing parameters (i.e. response range and sensitivity). We report on the design of allosterically-regulated DNA-based sensing systems that combine both sensing and regulatory domains within one molecule. This yields structurally simple molecular sensors with uncompromised specificity towards target and capability to tailor response range and sensitivity to a defined interval via rational manipulations of the regulatory domain. In particular, we demonstrated that incorporation of quadruplex structures into molecular beacons allows tuning both range of detectable analyte concentrations and response sensitivity with respect to the target analyte (i.e. ssDNA). We characterized the corresponding binding transitions via multiple techniques (PAGE, fluorescence, CD, UV) and showed that the design does not alter specificity of target binding and can be used to fine-tune aforementioned binding parameters. The general design principles presented in this report can also be applied towards variety of other potential targets (e.g. proteins, ions, RNAs, etc.).

Keywords: Bioanalytical, Biosensors, Nanotechnology, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Sensors
Facilitating the Detection of Informative Multiplexed MicroRNA Panels Using Silicon Photonic Microring Resonator Arrays

The methods used to study microRNAs (miRNAs) involved in cancer have failed to keep pace with our understanding of cancer pathology and are imperfect fits for the clinic. qRT-PCR, the current gold standard, is incredibly sensitive, relatively rapid, and cost effective, but only able to quantitate one target per assay. Conversely, microarrays are multiplexable, but slow and expensive. As a result, there are no clinically-adopted technologies for the multiplexed analysis of disease-relevant miRNAs. Here we show that a technology combining silicon photonic microring resonators and asymmetric polymerase chain reaction (aPCR) can fill these voids by simultaneously quantitating 25 miRNAs relevant to glioblastoma multiforme (GBM) and show that these results correlate well with qRT-PCR.

While multiplexed diagnostic panels have emerged in multiple tumor types, biomarkers for brain tumors have lagged behind. We use GBM, an extremely invasive glioma subtype that carries a mean survival time of 12-14 months, as a model system. While mRNA and protein profiling have helped identify underlying GBM biology, survival outcomes and therapeutic strategies have not changed greatly in the past few decades. As a result, researchers have turned to miRNA as a potential solution. In doing so, multiplexed miRNA biopanels have emerged that are predictive of GBM grade, recurrence, and survival. This abstract displays a validated microring resonator technology that is easily translatable to the clinic and facilitates the detection of GBM-specific meso-plex miRNA biomarker panels.

This work is supported by the National Institutes of Health and the NIH-sponsored Midwest Cancer Nanotechnology Training Center.

Keywords: Nucleic Acids, Array Detectors, Biosensors, Biotechnology
Application Code: Biomedical
Methodology Code: Sensors
Bacterial protein toxins secreted by pathogenic bacteria such as Cholera toxin can be lethal, pose threats to public health, and may potentially be used as biological weapons. Thus, sensors are needed for specific and sensitive detection of bacterial toxins. Existing methods mostly rely on analyte labeling (e.g. fluorescent labels) or suffer from non-specific interferences (e.g. SPR methods). In this work, we present a novel pull-down assay for the detection of Cholera toxin binding unit (CTB), based on the specific binding interaction between CTB and its cell membrane receptor, ganglioside GM1, combined with label-free mass spectrometry identification. GM1 was incorporated into 200 nm phospholipid vesicles composed of an NH\[sub\]2[/sub]-functionlized lipid and a polymerizable lipid (Bis-Den PC or Bis-sorb PC). Polymerization of the lipid formed crosslinking structures within the lamellar region of the vesicles, greatly enhancing vesicle stability. The polymerized, GM1- and NH\[sub\]2[/sub]-functionalized vesicles were covalently immobilized on sulfonate-modified, micrometer-sized silica particles, making novel vesicle-shell, silica-core particles. The core-shell particles were incubated with CTB, allowing for specific binding between CTB and GM1. The particles were pulled down by centrifugation for the pre-concentration of CTB. Finally, label-free CTB can be detected by either directly exposing the particles to MALDI-MS or releasing with protein denaturing solution, followed by ESI-MS analysis. Polymerization provides the vesicles with enough stability to survive centrifugation and MS detection conditions. The pull-down pre-concentration step and the large surface area of the vesicles contribute to the sensitivity of the assay.
Environmental contamination by heavy metal ions is a worldwide problem however current detection of hazardous metal ions requires expensive, laboratory-based instruments that may not be available to all analytical groups. New, low-cost techniques for the remote sensing of these ions is therefore desirable. Surface Enhanced Raman Scattering (SERS) is a highly sensitive, inexpensive technique that can be used for the multiplexed detection of metal ions. One of the main advantages of this technique is the potential for remote sensing due to the widespread availability of portable Raman spectrometers.

However, since metal ions lack a vibrational signature, reporter molecules are required in order to provide a spectral profile. In this work, 2,2-bipyridyl (bipy) is used as a universal ligand to coordinate to the metal ions Zn(II), Ni(II), Fe(II), Cd(II), Cu(II) and Cr(III). Each target ion was added to a solution of bipy and left overnight to allow complexation to occur. The complexes were then added to silver nanoparticles and the SERS response was measured. It was found that, when bipy was complexed to metal ions, changes in the resultant SERS spectrum occurred. Characteristic peak shifts and intensity changes were observed which allowed for unambiguous discrimination between each metal ion. Thus it was demonstrated that reporter ligands that strongly interact with multiple metal ions can be used to discriminate between these species, based on the specific spectral “fingerprints” that are produced.

This has the potential to be used in environmental monitoring, where the rapid, on-site detection of metal ions is necessary.

Keywords: Detection, Environmental Analysis, Metals, Surface Enhanced Raman
Application Code: Environmental
Methodology Code: Vibrational Spectroscopy
Determining the composition of mixtures of solids is a challenge in commerce. Typically, a product resulting from the refinement of plant material requires purity determination. Conversely, a natural commodity may be enriched by blending another ingredient to meet a nutritional or other performance chemically defined specifications. In quantitative near infrared chemical imaging, the qualitative identity of individual pixels in the field of view reveals what is present at each x, y location. How much is present depends upon the Absorbance of the chemical species of interest at its indicator wavelength. Binary pixel counting is refined by PLS data treatment to yield useful, reliable numerical quantitation.
An external cavity quantum cascade laser (EC-QCL) was applied in the photoacoustic detection of solid samples. Tunable lasers offer some advantages in spectral density and resolution when compared to the traditional Fourier transform infrared (FTIR) spectrometers. Typically, EC-QCLs have been used in trace gas analysis, but the tuning ranges have been dramatically increased enabling also the analysis of the solid samples that generally have much broader infrared peaks.

The high spectral power density of the EC-QCL was combined with an extremely sensitive optical cantilever microphone of the photoacoustic detector to achieve an ultimate sensitivity [1]. The EC-QCL used has a broad tuning range of 676 cm\(^{-1}\) (970–1646 cm\(^{-1}\)) in the mid-infrared region, which enables accurate broadband spectroscopy of large molecules. The latest designs offer a tuning range for over 1000 cm\(^{-1}\) in the pulsed mode in the mid-IR region. Also, some laser manufacturers nowadays offer fast sweeping of the laser, where the full tuning range can be scanned in seconds.

The carbon black, polyethylene, and hair fiber samples were measured with the EC-QCL photoacoustic detection using electrical amplitude modulation to demonstrate the possibilities of the setup. The same measurements were repeated with an FTIR spectrometer combined with a photoacoustic detector for a comparison. The EC-QCL photoacoustic setup yielded roughly an order of magnitude better signal-to-noise ratios than the FTIR setup with the same measurement time. The compact EC-QCL source and photoacoustic cell can be combined as a powerful and robust analyzer for solid samples in the future.


**Keywords:** FTIR, Laser, Photoacoustic, Spectroscopy

**Application Code:** Process Analytical Chemistry

**Methodology Code:** Vibrational Spectroscopy
Evolved Gas Analysis (EGA) is the perfect tool for characterizing the thermal behavior of organic and inorganic solids or liquids in more detail and elucidating the chemistry behind the processes under investigation. Thermogravimetric Analysis (TGA) is often coupled to FTIR as a means of generating more complete thermal characterization of the material under investigation. The TGA gives information on the temperatures at which various gases are evolved, the quantities of those components in terms of mass change, and also the rate at which the gases are evolved. Combining an FTIR into the output gas stream enables identification of the compounds generated in the gas phase. When properly configured, the data between the two instruments can be directly correlated in terms of time and temperature giving a more complete picture of the material's thermal breakdown processes. There exist two ways to connect an FTIR to the evolved gas stream of the TGA; the oldest and most established is connection between the two instruments by a heated capillary tube and the newest method involves integrating the FTIR directly on top of the TGA furnace, eliminating need for a heated transfer line. This lecture will compare and contrast the differences between these two TGA-FTIR coupling techniques, identifying the advantages and disadvantages to both.

Keywords: FTIR, Polymers & Plastics, Rubber, Thermal Analysis
Application Code: General Interest
Methodology Code: Vibrational Spectroscopy
Infrared spectroscopy is widely adopted as a technique for the identification of materials. Infrared microscopy allows spectra to be measured on small samples down to a limit of a few microns, with this limit dictated by the diffraction limit of the infrared radiation. Spectra can be measured on an IR microscope using transmission, reflectance or ATR techniques.

An infrared microscopy experiment can consist of multiple measurements on a sample at multiple points on the sample and the regions of interest (ROI) will almost certainly be of different sizes and shapes. Optimisation of the infrared measurement at each point requires an aperture to be set to fit within the ROI to avoid stray light or spectral interferences from adjacent materials or sample matrix. The experiment can also generate significant amounts of data that needs to be interpreted to get the required information from the sample. Manual evaluation of the data can add significant time to the sample analysis.

An infrared microscope system is described here that offers significant speed advantages over conventional infrared microscopes by utilising both hardware and software automation to streamline the infrared microscopy experiment workflow. New and innovative software algorithms for ROI detection can be applied to a wide range of different types of infrared microscopy experiments. Automation of data collection and data analysis provides answers in a fraction of the time required for manual data interpretation. Examples of applications of infrared microscopy for a range of industries will be presented.

Keywords: FTIR, Microspectroscopy, Molecular Spectroscopy, Software
Application Code: General Interest
Methodology Code: Vibrational Spectroscopy
Interfacial properties are largely determined by molecular structures at buried interfaces. Structure-property relationships at buried polymer interfaces, however, are not well understood due to a lack of non-destructive interface sensitive analytical techniques. This research focuses on the development of the nonlinear optical spectroscopic technique infrared-visible sum frequency generation (SFG) vibrational spectroscopy into a metrology tool for non-destructive characterization of molecular structures at buried metal/polymer and semiconductor/polymer interfaces in situ. Sample geometries for selectively detecting SFG signal generated at buried metal/polymer interfaces were studied which enabled characterization of molecular structure at the buried interfaces. Data analysis methodology for SFG signal generated from silicon-supported polymer thin films was also studied and the molecular structure at the surface and buried interface of the films was deduced. Functional groups such as methyl and phenyl groups were found to be ordered at both metal/polymer and semiconductor/polymer interfaces and interfacial molecular ordering was correlated with interfacial properties such as interfacial adhesion strength, moisture uptake, and dielectric breakdown.
Remote Condition Monitoring – What Can You Do When You Take the FTIR to the Samples?

This presentation will focus on what can be accomplished when you take a portable FTIR to remote locations for oil sampling for condition monitoring. This discussion will include the quality of the FTIR data, what parameters can be tested at the site to pick up early failures and how this data can be quickly uploaded to a lab for quick evaluation and interpretation. The data can be interpreted on the site by technical personnel, utilize a known response to conditions or send the data to a qualified lab for proper interpretation. This opens-up a new realm of condition monitoring.
Although protein dynamics are increasingly evoked as contributors to proteins function, rigorous experimental assessment of their role has been challenged by both the spatial heterogeneity of proteins and the rapid interconversion of potentially important conformational states. One approach to overcome these challenges is to combine the inherent high temporal resolution of infrared (IR) spectroscopy with the spatial resolution afforded by site-selective incorporation of vibrational reporter groups via protein engineering to generate a spatially and temporally complete picture of the conformational dynamics of proteins. This approach was applied to study the molecular recognition of the Src homology 3 (SH3) domain of the yeast protein Sho1 and a peptide containing the polyproline sequence of its physiological binding partner Pbs2. Carbon-deuterium bonds were used as strictly non-perturbative probes of the conformational changes involved in SH3-Pbs2 peptide molecular recognition. The spectral data reveal that formation of the bound complex leads to the population of multiple conformational states and suggest an induced-fit mechanism of molecular recognition. The site-selective introduction of the vibrational probes permitted a spatially resolved view of rapidly interconverting states involved in the mechanism of molecular recognition between the SH3 domain and polyproline ligand.

**Keywords:** Biospectroscopy, FTIR, Protein, Vibrational Spectroscopy

**Application Code:** Bioanalytical

**Methodology Code:** Biospectroscopy
The long-term stability of amorphous pharmaceutical formulations can be negatively impacted by undesired crystallization of the active pharmaceutical ingredient. In previous efforts, we have demonstrated detection limits in the ppb range for crystalline APIs within amorphous formulations by second harmonic generation (SHG) imaging, which is exquisitely sensitive and selective for crystallinity of chiral compounds. However, SHG alone provides little direct chemical information. In this study, SHG microscopy was integrated into a synchrotron X-ray diffraction experimental hutch, allowing rapid identification of regions of interest by SHG for subsequent targeted XRD analysis. By pairing the dimensions of the X-ray beam with the dimensions of the regions of interest, the background X-ray scattering was greatly reduced, allowing high S/N detection by XRD. Using this approach, PXRD has been performed in models for pharmaceutical formulations with average crystalline content in the low PPM to PPB range. Results will also be presented for analysis of a commercial amorphous final dosage form, in which a substantial fraction of the API was found to be in a crystalline state with large batch-to-batch variability.
X-ray Analysis (Half Session)

Determination of Pu in Spent Nuclear Fuel - Results from Field Testing of High Resolution X-Ray (hiRX)

Plutonium determination is an essential aspect of safeguards operations of spent nuclear fuel reprocessing facilities. High resolution X-ray (hiRX) is a new technique based on monochromatic wavelength dispersive X-ray fluorescence (MWDXRF) which was developed for quantification of plutonium in spent fuel. hiRX is a highly sensitive and selective technique due to the use of doubly curved crystal optics (DCCs), which provide monochromatic excitation and detection, resulting in high signal-to-noise ratio for the analyte. A benchtop prototype hiRX instrument has been tested with actual spent fuel samples from a reprocessing environment. A custom-designed ultralow volume sample cell, which contains 5 microliters of liquid sample when filled with a pipette, was used in the field testing. Calibration was performed using aqueous standards containing plutonium and uranium, since uranium is the primary matrix element of spent fuel and may be present in amounts greater than 100:1 (U:Pu, g/L). Analytical results including accuracy, precision, and limit of detection (LOD) will be presented and compared with laboratory validation results. Linear response (>0.99) over nearly 4 orders of magnitude and LOD of 0.7 mg/L were demonstrated in the laboratory using strontium as a model element for plutonium. hiRX offers an alternative, nondestructive assay which can provide rapid, direct plutonium quantification in a simple to operate platform. These specific results demonstrate the potential of hiRX for more general analytical applications for sensitive and selective elemental analyses.

Keywords: Atomic Spectroscopy, Quantitative, Trace Analysis, X-ray Fluorescence
Application Code: Nuclear
Methodology Code: X-ray Techniques
X-ray Fluorescence (XRF) spectroscopy is a useful tool for identifying substances and confirming their identity with little or no sample preparation. New capabilities of the energy dispersive XRF analytical microscope (micro-XRF) enable the recording not only of spectra of small sand particles (as small as 50-100 microns) but also of hyperspectral images and the collection of average spectra over certain areas. Multivariate analysis (MVA) can produce chemical distributions of elements and/or material classification based on Principal Component Analysis (PCA), Partial Least Square Discriminative Analysis (PLS-DA), in particular, with association between elements that can aid in identifying bonded phases. The analysis of micro-XRF data of sands taken in different areas can be used to identify geographic locations.

The XGT-7200V XRF analytical microscope was used in this study. X-ray fluorescence spectrum of the materials strongly depends on X-ray optical system, sensitivity of the detector and accelerating voltage. In addition, the background from the substrate will contribute to the spectrum of the small pieces because the excitation X-ray penetrates through the material and interacts with the substrate.

We collected and analyzed spectra of sand from different locations (USA, Europe, Middle East) in the range of 1.00-40.96 keV (<400 spectra). Because only a few spectra have additional features in the energy range above 15 keV, spectra were truncated and analysis was done in the spectral range of 1.00—15 keV. The Standard FPM algorithm was used to calculate concentration of oxides in all samples. This set of concentration was used to build a data set for PCA and PLS-DA. Correlation between classification based on spectral analysis and concentration analysis will be shown. The data shows that MVA allows the differentiation of samples which have very similar spectra features (concentration profiles) and this approach may be useful for forensic investigation.

Keywords: Chemometrics, Forensic Chemistry, Statistical Data Analysis, X-ray Fluorescence
Application Code: Homeland Security/Forensics
Methodology Code: X-ray Techniques
Environmental regulations for pollution control and goals for green fuels with higher hydrogen, lower aromatics and low hetero-atoms were the key factors for development of new technologies for petroleum refineries. In India, Auto Fuel Vision & Policy 2025 gives a road map for cleaner fuels such as Euro-V equivalent petrol and diesel. Sulphur content in MS & HSD is being reduced continuously and there are various technologies available to refiners to reduce sulphur in transport fuels.

Removal of sulphur by adsorption process is an efficient and feasible technology that reduces cost for sulphur reduction in gasoline and diesel. Sulphur is removed through adsorption which takes place at lower temperature and pressure compared to hydrotreating technologies thereby significantly reduces operating cost. Further, solid adsorbent can be regenerated easily with retaining activity. Indian Oil has developed an adsorbent based on Zn/Ni/Al system for removal of sulphur compounds from diesel streams. For optimization of Ni/Zn/Al content in adsorbents and to remove sulphur to highest possible level from diesel, various analytical techniques XRD, XRF, XPS, TEM & TGA were used. Characterization of adsorbents for sulphur removal using these techniques at different stages provided valuable guidelines for optimization of adsorbent for sulphur removal.

Fresh, regenerated and spent adsorbents were characterized by analytical techniques and mechanism was proposed. Fresh adsorbent contains oxides of Ni/Zn/Al while spent adsorbent shows significant amounts metal sulphides as revealed by XPS & XRD. Regenerated adsorbent shows reduction in sulphur indicating removal of sulphur upon regeneration as confirmed by XRD which shows absence of metal sulphides. TEM with EDX provided critical information about various phases. Based on analytical inputs, the composition of adsorbent was optimized for effective removal of sulphur from diesel.

Keywords: Fuels\Energy\Petrochemical, Materials Characterization, Sulfur, X-ray Diffraction
Application Code: Fuels, Energy and Petrochemical
Methodology Code: X-ray Techniques
Despite the efficacy of tamoxifen (TAM) in treating breast cancer patients, many women still relapse after long-term therapy. Therefore, it seems imperative to search for alternative remedies. This study investigated the antitumor effect of TAM, caffeic acid phenethyl ester (CAPE) and their combination in MCF-7 cells and tumor bearing mice. Upon combining TAM with CAPE, they exerted a strong cytotoxic effect against MCF-7 cells with IC50 of 4 µM compared to 20 µM for TAM and 10 µM for CAPE. Induction of apoptotic machinery and DNA fragmentation were accompanied by a down-regulation of Bcl-2 and Beclin 1 expression levels. However, microtubule-associated protein light chain 3-II levels were not altered. A time dependent decrease in vascular endothelial growth factor levels was also evidenced. The in vitro effect of this combination was recapitulate in the in vivo model by enhancing the percent increase in life span of mice, and inducing marked regression in size and weight of solid tumor. Conclusively, the anticancer effect of TAM and CAPE is mediated via attenuation of autophagy and elevation of apoptotic and angiostatic potentials. The ability of CAPE to reduce the effective dose of TAM provides the rationale for investigating this combination in preclinical models.

Acknowledgments
We thank the Faculty of Pharmacy, Cairo University and National Cancer Institute, Cairo, Egypt for financial support in the form of a Major Research Grant.
Both digitoxin and digoxin are widely prescribed for treatment of congestive heart failure. They are hardly distinguished by an immunoassay, in spite of the fact that digoxin is a metabolite of digitoxin. The present work reports a rapid LC-MS/MS method for accurate determination of digitoxin and digoxin in biological fluids. In the method, the two analytes as well as their interferences are rapidly (<5 min) resolved on a monodisperse sub-2 μm particle column and then detected with their specific MRM transitions. Significant ion suppression along with poor accuracy was initially observed due to the presence of high abundance of phospholipids in plasma and serum. These issues have been overcome by using a one-step procedure for simultaneous protein precipitation and phospholipid removal. The final method utilizes a high throughput sample preparation technique to minimize matrix effects using 96-well plate packed with proprietary zirconia sorbents. The result is a rapid and accurate LC-MS/MS determination of digoxin and digitoxin that provides an improved alternative to existing immunoassay methodologies.

Keywords: Liquid Chromatography, Mass Spectrometry
Application Code: Clinical/Toxicology
Methodology Code: Liquid Chromatography/Mass Spectrometry
The Use of Single Particle-ICP-MS in Nanomedicine and Drug Delivery Systems

Single Particle-ICP-MS is a new operating mode in ICP-MS that is dedicated to the analysis of engineered nanoparticles (ENPs) commonly used in Nanomedicine and drug delivery systems. Single Particle (SP-ICP-MS) allows the differentiation between ionic and particulate signals, quantitation of both ionic and particulate fractions, measurement of particle composition, concentration (part/mL), particle size, and size distribution, and assists in monitoring agglomeration.

The present work explores the use of SP-ICP-MS as a metrology tool for the analysis of engineered nanoparticles in biological matrices (blood, urine). Preliminary results show that Au ENPs (30 and 60 nm) are stable in diluted blood and urine. No change in the size of Au ENPs and/or concentration was noticed during the 24h study period. SP-ICP-MS is rapidly becoming a key analytical instrument in assessing the fate, behavior and distribution of (ENPs) in several types of matrices1,2 including biological fluids such blood and urine.

Reference:

Keywords: Biological Samples, Clinical/Toxicology, Nanotechnology, Toxicology
Application Code: Clinical/Toxicology
Methodology Code: Mass Spectrometry
Method for Assessing Residual Trace Metal Contamination in Acid-Washed Vials

Metal-Free Vial Analysis is an in-house developed method used to measure the residual contamination levels of trace metals in vials that have been acid washed by a third party contractor. Ten randomly selected vials are sent to our lab from each lot of 1,000 washed vials. Each of the vials is analyzed for aluminum (Al), chromium (Cr), manganese (Mn), copper (Cu), zinc (Zn), and nickel (Ni) contamination. Two milliliters of 1% nitric acid is added to each 6 mL vial and allowed to leach the insides of the vial for a minimum of one hour. Analysis of the leachate determines the cleanliness of each vial. Leachate from each vial must contain less than 0.1 ng/mL of Cr and Mn, <1 ng/mL of Ni, <5 ng/mL of Al and Cu, and < 50 ng/mL of Zn for the lot to be deemed acceptable for use in our clinical laboratory.

The validation of this method was performed using blinded analysis of vials that had been doped with NIST 1640 and 1643e water to attain levels near the rejection limit for each analyte. The NIST material was dried in previously washed vials. 1% nitric acid was then added to leach the insides of the vial, per procedure. Recoveries of the doped analytes were within 20% of the target values. Accuracy and recovery experiments demonstrated that the calibration is linear 20 to 100x the highest calibrator. The limit of detection was shown to be well below the rejection threshold for each of the elements. The validation demonstrated that this method is capable of detecting levels of contamination in a vial that would adversely affect patient results. This process ensures that serum and urine samples transported in the vials we provide to our clients are free of contamination from the vials.

Keywords: Clinical Chemistry, Mass Spectrometry, Metals
Application Code: Clinical/Toxicology
Methodology Code: Mass Spectrometry
An electrochemical sensor based on arginine (Arg) functionalized graphene (Arg-G) nanocomposite was fabricated for sensitive detection of acetaminophen. The nanocomposite was characterized by transmission electron microscopy (TEM), Raman spectra etc. The electrochemical behaviors of acetaminophen on Arg-G composite film modified glassy carbon electrode (GCE) were investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The experimental results indicated that the incorporation of arginine and graphene greatly enhanced the electrochemical response of acetaminophen. This fabricated sensor displayed excellent analytical performance for acetaminophen detection over a range from 0.1 to 100 μmol L⁻¹ with a detection limit of 0.05 μmol L⁻¹ (S/N=3). Moreover, the proposed electrochemical sensor also exhibited good reproducibility and stability, and has been used to detect acetaminophen in tablets with satisfactory results.

**Keywords:** Electrochemistry, Electrodes, Materials Science

**Application Code:** Clinical/Toxicology

**Methodology Code:** Electrochemistry
Adiponectin is a primary endocrine hormone secreted from adipocytes, and its level in blood is an important indicator of diabetes, obesity, and other metabolic diseases. Recent observations have shown acute adiponectin secretion in response to nutrient and hormone signals, implying the existence of a readily-releasable pool of vesicles. Unfortunately, tools to investigate dynamics of adiponectin secretion are lacking. We have previously developed microfluidic perfusion systems for primary adipocyte culture and sampling, yet adiponectin secretion could only be observed over the 1-hour time scale due to insufficient assay platforms. For this reason, we have developed a proximity ligation assay (PLA) to quantify adiponectin in smaller volume samples. This way, microfluidic secretion sampling can offer improved temporal resolution, and the adiponectin PLA can be used as the readout. Consistent with adiponectin's homomultimer structures, we observed the greatest assay response when employing the same antibody with two different oligonucleotide signaling arms, with a current limit of detection of 30 fmol and a dynamic range from 30 – 6 600 fmol (see figure). The assay also showed higher sensitivity toward multimers of higher molecular weight (mixture of 12-36 monomers) compared to trimers (figure inset). At this stage, assay sensitivity needs improvement to be useful for serum samples or small volume secretory samples. Future work will focus on the length and connectivity of the oligonucleotide arm sequences to enhance performance.
Clinical Chemistry

A New SRM for Assessment of Arsenic Exposure Through Urine Analysis

NIST’s Chemical Sciences Division (CSD) in the Material Measurement Laboratory and the Centers for Disease Control and Prevention (CDC) Division of Laboratory Sciences began a collaborative effort in 2004 to develop Standard Reference Materials (SRMs) for arsenic species in frozen human urine. Arsenic is a toxic element and a known carcinogen. The element is present in food and water in chemical forms that have varying degrees of toxicity. Toxicological evaluation of arsenic exposure must be based on the mass fractions of arsenic species rather than total arsenic. SRM 3669 Arsenic Species in Frozen Human Urine (Elevated Levels) is jointly developed by NIST and CDC. The SRM is intended for use in evaluating the measurement accuracy of arsenite (AsIII), arsenate (AsV), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and arsenobetaine (AB) in patients experienced acute arsenic exposure. SRM 3669 is related to SRM 2669 Arsenic Species in Frozen Human Urine that was introduced in 2009 for method validation in biomonitoring measurements of the general population. Whereas the target levels of arsenic species in SRM 2669 are approximately 50 to 95 percentile of the distribution of the population, the target levels of arsenic species in SRM 3669 are > 95 percentile.

Value assignment of arsenic species in SRM 3669 was based on liquid chromatography inductively coupled plasma mass spectrometry (LC-ICP-MS) and ion chromatography ICP-MS measurements made at NIST, and LC-ICP-MS measurements made at CDC. Value assignment of total arsenic was based on ICP-MS measurements and radiochemical neutron activation analysis (RNAA) made at NIST.

Keywords: Chromatography, ICP-MS, Speciation, Standards
Application Code: Clinical/Toxicology
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Diversity of engineered nanoparticles (NPs) and their contribution to the development of technology and industry are remarkable. While NPs have enabled important technological advances, they might also have adverse impacts on human and environment. Therefore, novel detection techniques are needed in order to provide better understanding on how NPs interact with living organism. This presentation will describe the use of electrochemical microsensors for assessing localized in vivo nanotoxicity, oxidative stress, inflammation and organ dysfunction induced by NPs in intact zebrafish embryos. Electrochemical microsensors display real-time in vivo measurement capability of various biomarkers with high sensitivity and selectivity while providing high spatial and temporal resolution. Fabrication, characterization and in vivo application of an implantable electrochemical biosensor for the detection of physiological concentrations of nitric oxide (NO) in zebrafish embryos at organ level will be presented. The use of these sensors as quantitative tools for mechanistic investigation of nanotoxicity will be illustrated. Results of these assays and the potential of this method to determine biomarkers of organ abnormality and inflammation during NP exposure will be discussed.
An authentic science learning model through collaborative learning suitable for large urban public colleges with limited resources was proposed and implemented in the course of Instrumental Analysis (science major), Environmental Science (non-science major), and Undergraduate Research at John Jay College, the City University of New York. The goal of this project is to enhance student learning of science concepts and skills by introducing them to real world problems and solutions. Using sustainability-chemistry as an example, this project allowed students at different academic levels to work collaboratively on field-based environmental projects to investigate pollutants in Hudson River and other EPA Superfund sites in New York City, establish close communication among participants and mentors, and develop positive attitudes towards science learning, and cultivate social awareness.

Keywords: Education, Environmental Analysis
Application Code: Other
Methodology Code: Education/Teaching
Hands on learning in STEM courses is essential. This style of learning is seen quite frequently in the field of chemistry where nearly every undergraduate lecture has a corresponding laboratory course, and a variety of instruments are routinely used to make measurements. With growing undergraduate enrollments larger numbers of students are filling instructional chemistry labs, and the demand for a sufficient number of instruments can be a financial obstacle for many chemistry laboratory coordinators. To alleviate the cost of purchasing large numbers of commercial systems a push has begun to develop low-cost alternatives. In this presentation, a low-cost Raman spectrometer which utilizes a Canon Rebel T2i digital SLR camera as the detector is presented. Similar Raman teaching instruments employ miniature USB type spectrometers as detectors which typically cost $2,000 - $3,000. In comparison, the digital camera spectrometer presented here can be built at a cost of $750 - $1,000. This type of instrument is ideal for teaching. The open configuration allows for visual inspection of all optical components (reducing the black box effect) and requires manual adjustment of instrument parameters. The system has collected quality Raman spectra of various solids in 100 seconds or less. In addition, spectroscopy topics such as slit width, spectral resolution, signal to noise, signal averaging, and signal smoothing have been demonstrated using the instrument. Furthermore, the instrument can be quickly converted to a visible spectrometer with the addition of a light source and changing to a less dispersive grating for Beer’s law type experiments.

Keywords: Raman, Spectrometer, Spectroscopy, Teaching/Education
Application Code: General Interest
Methodology Code: Education/Teaching
The development of laboratory experiences based on pharmaceutical processes has become increasingly important in the undergraduate curriculum. Adaption of current research techniques for the undergraduate laboratory is vital in order to introduce students to concepts of interest to the scientific community. This allows undergraduate students to become acquainted with a variety of topics with hopes they will find their niche in the chemistry world more readily. A study of the diffusion of drugs through a hydrogel membrane can be used to provide students with experiences in chemical kinetics and polymer application in pharmaceutical sciences. Hydrogels are hydrophilic material created by polymerization of monomers to form a cross-linked material and are able to absorb large amounts of water, making them the perfect drug delivery system. Since water has the ability to enter this system, they are capable of absorbing water soluble molecules. PNIPA hydrogels were formed using N-isopropylacrilamide as a monomer, N,N'-methylenebisdiaacrylamide as a cross-linker and sodium disulphite as a polymer initiator. Hydrogels were synthesized, dehydrated, and then reconstituted using a solution of triamterene and aminophylline dissolved in water. PNIPA drug loaded hydrogels were placed in a warm water bath in order to facilitate the release of the solution. Water samples were analyzed at specific time points using Ultra-Violet Visible (UV-Vis) spectroscopy to monitor the release of triamterene and aminophylline.
A well known and interesting experiment named as Iodine Clock Reaction (ICR) has already been used at graduate and under graduate level since last several years, to demonstrate the function of temperature on the rate of reaction. We present here a double clock kinetics experiment based on the repeated oxidation of iodide by BrO3- followed by H2O2. The primarily aim of this work is to enhance the scope of ICR experiment by some modification and extension in the existing experimental conditions. The proposed Iodine Double clock Reaction (IDR) has been successfully employed on the determination of the activation energies of two successive reactions in a single experimental setup. The obtained values of activation energies are 24.07 KJ mol-1 and 33.63 KJ mol-1 and Arrhenius factors are 2.65 x 105 and 117.57 x 105 for BrO3- and H2O2 respectively. The obtained data will be helpful to put forward the comparative reaction mechanisms of both the reactions.
Research focusing on assessment of abandoned mine drainage (AMD) from the old Marchand coal mine, and remediated at the Lowber AMD treatment facility in southwest Pennsylvania, will be presented and discussed. This study is sponsored by the Rho Theta Chapter of the Gamma Sigma Epsilon National Chemistry Honor Society and in collaboration with the Sewickley Creek Watershed Association (SCWA). AMD is perhaps the most prevalent form of water pollution in southwest Pennsylvania, due to the abundance of abandoned coal mines that accumulate ground and surface water over time. This water eventually reaches the surface and produces insoluble hydrated iron(III) oxides that may contaminate streams and render well water unfit for human consumption. The goals of this research are: (1) to assess selected analytes in AMD from the Lowber facility in the field and laboratory, (2) to assist the SCWA in more frequent evaluation of AMD, and (3) to develop remediation strategies for use at Lowber and other potential sites in the Sewickley Creek Watershed.

Determination of such analytes as alkalinity, acidity, pH, sulfate, chloride, total suspended and total dissolved solids, and metals such as iron, aluminum, manganese, and calcium will be performed by appropriate methods. Sample collection and analytical methodologies will be presented and discussed, as will results of determinations performed to date and

Keywords: Education, Environmental Analysis, Spectroscopy, Titration
Application Code: Environmental
Methodology Code: Education/Teaching
Abstract Text
The effectiveness of using videos that undergraduate students could view prior to conducting their experiments in an Analytical Chemistry laboratory course was examined. To this end, we developed instructional videos to show students how to conduct the experiment. Developing a video (lasting 5-10 minutes) for each weekly experiment typically required several days to complete. Filming an experiment required a few hours, but then more time was required to condense and annotate the material. Undergraduate students were required to download the video and view it before the laboratory class commenced. As a result, the instruction time on how to perform the experiment was significantly shortened, thereby allowing more laboratory time. Furthermore, the response to these videos was very positive, with students enjoying the visual impact. Here, we describe our experience in this endeavor.

Keywords: Education, Laboratory, Teaching/Education
Application Code: Safety
Methodology Code: Education/Teaching
In 2014 OSHA implemented new standards (29CFR1910.1200) regarding Classification and Labeling (C&L). This new C&L standard brings the United States in line with the United Nations Globally Harmonized System (GHS). This standard system of labeling applies to hazardous goods shipped and sold in the United States and globally. The new requirements apply to Safety Data Sheets (SDS) and product labels for all chemicals classified as “hazardous” under the OSHA guidelines. They are required to be implemented by all manufacturers, importers and distributors of hazardous goods. By 2015 all suppliers of hazardous goods must be in compliance with the regulation.

The poster will examine the main requirements for GHS compliant hazardous chemicals. The largest of these changes will affect the SDS and product labels. The GHS implementation applies to all levels of the company, and across various departments such as QC/QA, Research and Development, Method Development and Health Safety and Environmental. The GHS labeling requirements apply to all chemicals used in the lab from vials and bottles to cans and drums. The information presented will educate the user on the changes to the law so they will be prepared for the changes.

Keywords: Chemical, Sample & Data Management, Sample Handling/Automation, Standards

Application Code: Regulatory
Methodology Code: Education/Teaching
In order to make accurate analytical measurements, the calibration design must be optimized. Calibration designs are used to aid in determination of an unknown. The optimal calibration design had not been previously determined in which error is minimized. Both absorption and emission have noise sources that affect the optimum region for measurements and the optimal design will be dependent upon the type of limiting noise.

The major source of error is detector noise, which obscures and degrades the ability to interpret the response. Random instrumental noise in spectrophotometric measurements can be divided into three classes: (1) sources that are completely independent of response; (2) sources with variance that is directly proportional to the response; and (3) sources of noise with variances that are related to the square of the response. The magnitudes of these noise sources affect the signal-to-noise ratio of measurements.

This study examined the design of calibration strategies which optimize precision of UV-visible absorption spectroscopy and inductively coupled plasma-optical emission spectrometry measurements. Different calibration strategies, composed of different concentrations and number of replicates, have been evaluated to try to determine the calibration design that will minimize imprecision as measured by the average relative concentration error integrated over the entire calibration graph.

Our research indicates that the range of calibration imprecision varies over a range of 10:1 for ultraviolet-visible absorption and 197:1 for inductively coupled plasma-optical emission spectrometry depending on the location of the standards. This information allows us to conclude that some calibration designs are superior.
The concepts of Analytical Method Transfer (AMT) have been integrated in the undergraduate analytical chemistry curriculum at St. John Fisher College (SJFC) and Kennesaw State University (KSU). Students and faculty at SJFC and KSU are developing and validating analytical laboratory methods that are focused on teaching students AMT. The laboratory methods are dissolution testing and HPLC analysis of glucosamine tablets, dissolution and flame atomic absorption spectroscopy of potassium gluconate, and dissolution testing and UV analysis of ibuprofen tablets. These analytical methods are being transferred to the other school to confirm the validation - hence perform an AMT as found in the real world. This transfer will return back to the original school to see if the next group can complete the same validation and accomplish the method transfer. Once they have been validated, POGIL-based lecture and lab exercises using our data to be tested at other schools will be written. The POGIL exercises will walk the students at other schools through the steps of AMT, enabling them to determine whether the transfer was successful or not. We aim to improve student engagement and learning in analytical courses at both schools and to disseminate POGIL-based lecture and lab activities for use at institutions that lack dissolution testers to compare learning gains between classes that have the instrumentation and those that do not. We gratefully acknowledge the financial support of the NSF (DUE: 1141021 and 1141042) for this project.
Abstract Text
Safe handling of hazardous substances cannot be ensured without safely information. The most common means for providing information are the Material Safety Data Sheets (MSDS), and labels. The MSDS are important reference for health and safety information for many chemicals. Container labels provide the basic information that a person needs to know to handle a particular product safely, they show how a chemical dangerous it is, it explains what to wear when using the chemicals, also shows directions for immediate first aid. Also valuable is the National Fire Protection Association labeling system, which shows the type and the degree of a chemical hazard.

There are a number of smartphone Apps which are good resources to find information concerning the hazards of many chemicals, the MSDS and labels. The use of these Apps in the chemistry lab serves as a quick reference and educational tool for students. This paper is based on an ongoing research program examining the implementation of smartphones Apps to help students develop personal safety awareness. Our objective is to explore how students can take advantage of these Apps to react logically to a situation and keep people safe.

There are more than 300 Apps for chemistry. They cover all concepts of chemistry from the basic of naming compounds and writing formulas to more advanced topics like the interpretation of spectroscopy data. Students learn that every chemical is dangerous; chemicals must be handled safely in order to avoid skin, eye or inhalation exposure. Most students made positive comments about what they thought of the use of Apps for learning how to handle hazardous chemicals.

Keywords: Chemical, Computers, Environmental, Teaching/Education
Application Code: Environmental
Methodology Code: Education/Teaching
**Improving Confidence and Perseverance in a Laboratory Project**

The impact of a half-semester laboratory project in Analytical Chemistry on student attitudes towards laboratory work was assessed. The laboratory project tasked students with working in small groups to recreate a literature paper. The project allowed students to make experimental design decisions beyond a typical guided laboratory experiment. Before performing the experiment, students submitted a detailed procedure including all calculations for preparation of the standards and samples, choice of glassware, and a data analysis plan. After experiment implementation, students submitted a written laboratory report. The attitude survey was administered at the beginning and end of the project. The 30 questions on the assessment were categorized. Improvement was shown in Confidence, Perseverance, and Value of Collaboration. For example, there was a 24% improvement in both understanding of the lab project and belief in reliability of their results. Minimal change was noted in Value of Work, Value of Learning, Communication and Reading Skills, and Future Plans.

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Susan Oxley  
St. Mary's University

**Keywords:** Education, Teaching/Education  
**Application Code:** General Interest  
**Methodology Code:** Education/Teaching
Persistent organic pollutants (POPs) are a group of chemicals that include brominated flame retardants (BFRs) and polychlorinated biphenyls (PCBs). These chemicals can be distributed throughout the environment through soil, water, and air contamination and once in the environment do not readily break down. Due to the lipophilic nature of these components they accumulate in the fatty tissue of animals and bioaccumulate up the food chain. According to the World Health Organization human breast milk is an ideal matrix to monitor the levels of POPs in not only the mother and infant, but also as a key indicator of the levels of these chemicals in the local environment.

Current methodology for the analysis of PCBs and BDEs can be expensive, solvent intense and time consuming. The QuEChERS extraction approach coupled to a silica cartridge SPE cleanup may be an attractive sample preparation alternative for biomonitoring efforts for halogenated POPs in milk. Comprehensive two-dimensional gas chromatography (GCxGC) with an electron capture detector (ECD) may also offer a more cost-effective alternative. Method development was done using whole cow milk and later compared to a NIST Standard Reference Material of Human Breast Milk.
Ammonia (NH3) is one of the most well-known odorous gas species in the atmosphere. Ammonia is frequently monitored for managing odorous pollution sources and air quality management. It is difficult to accurately measure ammonia concentration in the air due to its reactive, adsorptive, and unstable nature. For the accurate measurement, primary standard gas mixtures (PSMs) should be available to calibrate ammonia measuring instrument. PSMs are prepared in cylinders by gravimetric method in which high purity gas source or liquid sources are weighed. One of the major problems for preparing PSMs in cylinders, especially for reactive gases, is the stability of the target gases in cylinders. However, it is rare to find quantitative information on the extent of stability of \( \text{mol/mol NH}_3 \) in the highly pressurized gas cylinder. In this study, we prepared ammonia PSMs at 35 \( \text{umol/mol} \) in cylinders to evaluate the long-term stability of ammonia PSMs at 35 \( \text{umol/mol} \) level and compare the stability depending on different cylinder types. Ammonia PSMs at 35 \( \text{umol/mol} \) were prepared by three steps dilution from the high purity ammonia sources. Results from this study show that ammonia PSMs are consistent within their preparation uncertainties for eight years, although a decreasing trend is observed in PSMs prepared 2006.
### Abstract Text

Volatile organic compounds (VOCs) in the atmosphere have been determined to be a human risk factor in urban, suburban, and industrial environments. This study aims to characterize the distribution and concentrations of ambient air toxics in Memphis and identify major sources of VOCs. During four seasons in 2014, about 530 ambient air samples have been collected in various locations in Memphis and surrounding areas for the analysis of VOCs with the TO-15 method. GC-MS was used with a preconcentrator outfitted with a glass bead trap and a Tenax trap in cryogenic mode to analyze the VOCs in scan and/or SIM mode. The ambient air monitoring data showed that there were about 30 compounds that do not belong to the list of TO-15 target compounds that necessitate the preparation of calibration standard mixtures of these non-TO-15 compounds. These include cyclohexene, 3/4-chlorocyclohexene, cyclohexanone, C6-C10 aldehydes, dimethyldisulfide, dimethyltrisulfide, acetonitrile, and 2-cyclohexen-1-one. The improvement in the analytical sensitivity of the non-TO-15 compounds via SIM mode analysis of their fragment ions at specific m/z ratios will be presented. The comparison of non-TO-15 compounds in their concentration levels and emission sources will be highlighted in this project. Also, the Toxics Release Inventory of emission sources near the sampling sites were examined to correlate the air quality profiles with industry-specific chemical releases.

### Keywords:
- Air
- Data Analysis
- Gas Chromatography/Mass Spectrometry, GC-MS

### Application Code:
- Environmental

### Methodology Code:
- Gas Chromatography/Mass Spectrometry
A method aimed at improved analysis of 73 target volatile organic compounds (VOCs) at trace levels has been implemented for ambient air analysis in Memphis. Analytical instrumentation based on gas chromatography-mass spectrometry (GC-MS) coupled with a preconcentrator outfitted with a glass bead trap, a Tenax TA trap, and cryofocusing capability was used in accordance with the USEPA’s TO-15 protocol to achieve detection limits down to 0.1-1 ppbv for the 73 compounds. Method detection limits (MDLs) based on both separate scan mode and selected ion mode (SIM) as well as simultaneous scan and SIM modes were compared to give an indication of their relative sensitivity. Furthermore, the dependence of analytical sensitivity on the ions of different m/z values will be presented. The influence of the preconcentrator parameters on the chromatographic resolution and quantitative precision for closely eluting compounds was evaluated. Data analysis programs such as Automated Mass Deconvolution and Identification System (AMDIS), and Target View were used to resolve the spectra of overlapping chromatographic peaks in order to probe the identity of co-eluting VOCs in the samples. The comparison of quantitative accuracy of co-eluting compounds based on different selected ions for SIM mode analysis will be highlighted. The distribution of TO-15 target compounds detected at various sampling site in Memphis is explained in the context of industrial releases, mobile source emissions, and biogenic emissions.

Keywords: Environmental/Air, Gas Chromatography/Mass Spectrometry, Trace Analysis, Volatile Organic Compounds
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Environmental Chromatography

Fenceline Monitoring – Low PPM-Level BTEX Analysis in Air Using a Fast, Portable Micro Gas Chromatograph

The Environmental Protection Agency is about to propose mandatory monitoring of air concentrations at the fenceline of refinery facilities to ensure proposed standards are being met and that neighboring communities are not being exposed to unintended emissions. This will require the multi-point analysis of compounds that are potentially harmful for our environmental, such as benzene, toluene, ethyl benzene and xylenes.

This poster highlights fast analysis of BTEX compounds in air with detection down to low ppm levels using a micro gas chromatograph. A portable field case, equipped with rechargeable batteries and carrier gas, was used to enable on-the-spot measurements. Direct, on-site analyses secures the integrity of the sample. Moreover, it eliminates sample transportation to the lab and direct action can be taken when limits are exceeded.

The instruments small form factor (shoe-box size) allows it to be transported easily to multiple sample points on a refinery facility. Moreover, its low use of operational gases and power simplifies integration is mobile laboratories.

Keywords: Air, Environmental, Gas Chromatography, Portable Instruments
Application Code: Environmental
Methodology Code: Portable Instruments
US Environmental Protection Agency Methods TO-15 and TO-17 list a number of ‘air toxics’ that must be monitored in urban or industrial locations in order to minimise further ozone depletion and avoid negative health impacts. Due to their hazardous nature, it is vital to obtain comprehensive and sensitive data for an air sample in order to detect all compounds down to the lowest concentrations. Traditionally, the detection of trace-level compounds in a sample is performed by quadrupole MS in either full scan or selected ion monitoring (SIM) mode. However, qMS is a mass filter, with a high percentage of ions being wasted, thus limiting sensitivity. Moreover, in SIM mode, only target compounds can be monitored, meaning that full characterisation of the sample is not possible in a single run, and retrospective searching of data is limited. The use of time-of-flight mass spectrometry (TOF MS) can overcome this issue by providing highly sensitive detection whilst acquiring full-range mass spectra, to allow both target and unknown identification in a single, rapid analysis. Nevertheless, acceptance of TOF technology has been restricted due to large instrument size and the production of spectra exhibiting mass discrimination. This poster describes an innovative, robust TOF MS system that eliminates these problems. The generation of classical EI spectra allows traditional library searching for confident identification of trace-level compounds. The complementary use of GC–TOF MS with thermal desorption is detailed for the analysis of an urban air sample, by means of a cryogen-free, analytical system designed to offer flexibility of sampling methodology, fully compliant with US EPA Methods TO-15 (canisters) and TO-17 (sorbent tubes).
Thermal desorption (TD) coupled with gas chromatography is a common technique used for the analysis of volatile, semi-volatile and toxic organic compounds in air and soil vapors. Samples are collected by either passive sampling or active sampling by pumping the air sample through a thermal desorption tube packed with appropriate sorbents for the analytes of interest. The samples are subsequently analyzed by heating the sample tube while flowing a carrier gas through the tube to desorb the analytes then directing the outlet flow to a GC column for chromatographic analysis. The thermal desorption tubes can be reused many times but they need to be “conditioned” (or cleaned) prior to each sample collection. Conditioning is a process in which the tube is heated while flow of a high purity inert gas, such as He or N2, is applied to drive off any residual organic compounds adsorbed on the tube. The PerkinElmer TurboMatrix Automatic Thermal Desorber (ATD) can be coupled with a GC to automate the process of sample desorption analysis and conditioning for up to 50 thermal desorption tubes sequentially. The conditioning process generally takes 1-2 hours per tube and since the TurboMatrix ATD works sequentially it can take 50 – 100 hours to condition 50 TD tubes wasting valuable time on the ATD that could otherwise be used for sample analysis. The new PerkinElmer TurboMatrix TC-220 tube conditioner is an offline accessory that can be used to condition up to 20 TD tubes simultaneously which significantly reduces the time required for conditioning and frees up the ATD for analysis of samples. In this case study at Pace Analytical Services (Minneapolis, MN) we demonstrate the significant time savings as well as improvements in productivity by taking advantage of the TC-220 tube conditioner for offline conditioning as opposed to conditioning tubes on the ATD.
The focus on water quality in the last decade has increased demands on regulators to monitor and improve pollutant levels. Typical analytical protocols for semi-volatile organic pollutants in water include time-consuming and laborious extraction and enrichment steps, with liquid–liquid extraction being most common. The drive for improved water purity also demands ever-lower limits of detection.

Addressing these challenges, this poster will illustrate the simple and sensitive analysis of semi-volatile organic pollutants in water, by combining enrichment through high-capacity sorptive extraction with modern time-of-flight mass spectrometry (TOF MS). As well as preconcentrating water-borne pollutants, the new high-capacity sorptive extraction samplers are simple to use – they are introduced to the bulk sample, and then directly thermally desorbed, with no further sample preparation required.

Conventionally, trace-level detection of target compounds is often achieved using quadrupole GC–MS methods with selective ion monitoring (SIM), but this protocol prevents retrospective searching of data for contaminants of emerging concern. The poster will show how TOF MS can overcome this issue by providing highly sensitive detection whilst acquiring full-range reference-quality mass spectra, and so allow identification of targets and unknowns in a single, rapid analysis.

Keywords: Gas Chromatography/Mass Spectrometry, Sample Preparation, Semi-Volatiles, Water
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
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<td>Combining High-Capacity Sorptive Extraction and Bench-Top TOF MS in the Analysis of Personal Care Products</td>
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<tr>
<td>Primary Author</td>
<td>Nicola M. Watson</td>
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<td>Co-Author(s)</td>
<td>Caroline Widdowson, David Wevill, Helen Martin, Peter Grosshans, Steve Davies, Vanessa Frost-Barnes</td>
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**Abstract Text**

The fragrance of a personal care product plays a major part in marketing and customer satisfaction. Whilst the combination of fragrance compounds creates the unique, pleasing fragrance of a product, their concentrations can span several orders of magnitude in a single sample, and a number of them have been identified as suspected allergens. In addition, whether profiling the fragrance of a product or screening for suspected allergens, samples of shower gels, soaps and other cosmetics are not simple to handle, and typically require cumbersome preparation steps such as liquid–liquid extraction.

This poster will illustrate the application of Markes’ sampling, analysis and data-processing solutions to the simple and sensitive analysis of personal care products. With regard to sampling, Markes’ high-capacity sorptive extraction samplers provide a simple way of sampling semi-volatile fragrance compounds from complex, difficult matrices. The samplers are introduced to the bulk sample and then directly thermally desorbed, with no further sample preparation required.

Addressing the highly variable concentrations of fragrance compounds, the poster will then describe how quantitative re-collection and automated re-analysis with Markes’ thermal desorption (TD) technology facilitates repeat analysis with high and low splits, so extending the dynamic range with a single sample. Finally, the poster will describe how the high data rate of time-of-flight mass spectrometry (TOF MS) can allow fast analysis with detailed spectral information. This, combined with sophisticated data-processing algorithms, enables confident compound identification in complex matrices with high-speed chromatography.

**Keywords:** Gas Chromatography/Mass Spectrometry, Sample Preparation, Thermal Desorption, Time of Flight MS

**Application Code:** Consumer Products

**Methodology Code:** Sampling and Sample Preparation
Results from a Si-microfabricated GC X GC subsystem with each of two different microsensor-based detector technologies are presented. Point-of-care diagnostics or networked environmental sensing applications are envisioned. The separation module includes first- and second-dimension etched-Si columns and an etched-Si dual-stage µthermal modulator (µTM) on which we reported last year. Two novel detectors have now been explored: a 4-element µchemiresistor (CR) array employing sorptive nanoparticle interface layers; and a recently developed µoptofluidic ring resonator (µOFRR) employing a PDMS interface layer. The 1st dimension comprised two series-coupled µcolumn chips with a crosslinked PDMS phase. The 2nd-dimension µcolumn chip used a room-temperature ionic liquid (RTIL) phase. The dual-stage µTM chip had a crosslinked PDMS phase. On-chip heaters heated each modulator stage at ~ 2400 °C/s, and a thermoelectric cooler beneath cooled each stage to -20 °C between modulations. Previously the RTIL phase was too retentive for polar analytes, giving fwhm values up to 2.7 sec for heptanal with FID. This problem has been rectified. A 4 component separation with the CR array showed excessive broadening of modulated peaks due to slow desorption rates from the nanoparticle films and non-ideal flow dynamics in the cell; fwhm values were up to 8X higher than with FID. The effectiveness of a newly designed sensor array with improved fluidics (currently under test) will be demonstrated. Using the µOFRR, fwhm values of modulated peaks as low as 125 msec were obtained, on par with FID. An 11 component GC X GC separation was achieved with the µOFRR (see figure).
Localized surface plasmon resonance (LSPR) in films of thiolate- and dithianate-monolayer-protected gold nanoparticles (MPN) of different core sizes and monolayers probed at multiple visible wavelengths near the LSPR maximum has been exploited to achieve remarkable vapor selectivity from a single sensing film. Solvent cast or spin-coated multilayer films exhibit response selectivity arising from differences in sorption-induced increases in the interparticle distance and the refractive index (RI) of the ligand matrix, which are functions of ligand-vapor affinity, ligand-vapor RI differences, and metal core size. Films of MPNs with core diameters of 4, 5, 20, and 40 nm and 6 different thiolate or dithianate ligands were cast and their visible transmission spectra recorded before, during and after exposure to high concentrations of 6 individual VOCs. For a subset of VOC-MPN pairs we have generated linear calibration curves from 15-100% saturation. Absorbance shifts at 405, 532, and 630 nm were used to create response patterns for all MPN-VOC pairs. Analysis by Monte Carlo simulation coupled with principal components analysis indicated remarkably high degrees of VOC discrimination: 89-100% discrimination with a single 5-nm or 40-nm MPN film of one type. Arrays of just two 4- or 5-nm MPN films were also remarkably selective (> 95% discrimination) even with just a single wavelength response from each film. However, sensitivity is low and replicate films give highly variable responses. Current tests are assessing the influence of film ordering on reproducibility, exploring responses to binary mixtures, and using MPNs films on microfabricated optofluidic ring resonator ([micro]OFRR) sensors.
A displacement assay based on high-performance affinity chromatography (HPAC) with fluorescence detection was developed for the analysis of drugs in water and biological samples. This assay first involved the application of a labeled analog of the drug phenytoin onto an affinity column containing immobilized bovine serum albumin (BSA) as a binding agent. Samples were then applied to affinity column, resulting in displacement of the labeled phenytoin. One of the applications examined for this assay was for the detection of drugs and emerging contaminants in water. This assay provided detection ranging from low nM concentrations up to µM levels for drugs such as warfarin and carbamazepine. The results for tap water and water runoff samples that were spiked with these drugs showed good agreement with the actual concentration of these drugs. This method was also examined for use in measuring the free, or non-protein bound, fractions of drugs in biological samples. This format was used to examine the free drug fractions in drug/HSA samples containing warfarin, verapamil, or carbamazepine. The results were comparable to those predicted based on the known binding constants for these systems. The information provided by this study indicates that this type of assay can potentially be used as a potential screening tool for environmental contaminants and for the analysis of free drug concentrations in clinical samples.

**Keywords:** Bioanalytical, Environmental/Biological Samples, HPLC

**Application Code:** Bioanalytical

**Methodology Code:** Liquid Chromatography
The purpose of this research was to investigate the oxidizing capacity of chlorine dioxide for degrading various toxic or odorous organic compounds in both gas and liquid phases. This research project is useful for exploring the potential application of chlorine dioxide for remediation of contaminants in different environmental media. The gas phase reaction was monitored using a 10-meter gas cell and FTIR instrumentation that allows the measurement of reaction products and the chlorine dioxide at the parts-per-million levels. The GC-MS technique was used with a three-trap preconcentrator to characterize the reaction products with greater specificity at lower detection limits than the FTIR method. The degradation trends of five target compounds including cadaverine, cyclohexyl mercaptan, dimethyl trisulfide, methyl disulfide and 2,6-di-tert-butylpyridine were compared. The concentrations of cadaverine and cyclohexyl mercaptan were reduced most effectively. The major byproduct during the reaction between chlorine dioxide and cadaverine was ammonia. The reactions between chlorine dioxide and dimethyl trisulfide or methyl disulfide yielded byproducts that consisted of alkyl halide, sulfone, sulfonate, and sulfur dioxide. The sterically hindered 2,6-di-tert-butylpyridine was more of a challenge for its chemical degradation by chlorine dioxide and showed very slow reaction rate. The GC-MS analysis of the degradation studies via Tenax-based sorbent trap was found to yield confounding results because of the undesirable reaction of chlorine dioxide with the Tenax TA sorbent. The Tenax trap was replaced with a glass bead trap and the analytical results of both preconcentration approaches for achieving low detection limits will be presented.

The funding for this research was provided by the Department of Chemistry, Middle Tennessee State University.

Abstract Text

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The funding for this research was provided by the Department of Chemistry, Middle Tennessee State University.

Keywords: FTIR, Gas Chromatography/Mass Spectrometry
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Purpose: To develop a simple HPLC/CAD method for the determination of ETU in multiple formulations. The selectivity of the method enabled separation of ETU from a cellulose matrix and background interference from deionized water using a universal detector. This also enabled a simple processing procedure to be used.

Methods: A Dionex Ultimate 3000 HPLC coupled a Dionex Corona charged aerosol detector was used for the quantitation of deionized water formulations containing ETU. Chromatography was achieved with a YMC Pack ODS-AQ C18, 150 x 4.6 mm, 3 µm particle-size column (column temperature of 30°C) along with an isocratic elution using mobile phase 90:10 (v/v) 1% formic acid in deionized water: 1% formic acid in acetonitrile (0.5 mL/min). Data acquisition and analysis were performed using Dionex Chromeleon[registered] software version 6.8.

Results: With the HPLC/CAD method, linearity was demonstrated from 5.00 to 100 µg/mL. In addition, quality controls (0.0100, 1.00, and 3.00 mg/mL) showed inter-session variability of 1.5% to 6.8% RSD and inter-session accuracy ranging from -3.4% to 4.6% RE. The LOD of ETU was 0.5µg/mL and the LOQ of ETU was 5.00 µg/mL.

Conclusion: A simple and accurate method was developed and validated for the quantitation of ETU via HPLC/CAD. The method was used to quantify the amount of ETU in deionized water gavage formulations with separation from a cellulose matrix, as well as, background interference from deionized water, with a simple processing procedure.
Environmental Samples - Sampling, Detection and Water/Soil Samples

Automation of a Solid Phase Extraction (SPE) Fractionation Step for Aliphatics and Aromatics in Oil Fingerprinting Analysis

The unique chromatographic profiles of petroleum aliphatic and aromatic components are useful for determining oil spill sources, degree of weathering of the spill and the type of oil product released into the environment. These profiles are also useful in determining the petroleum depositional environment and source (petrogenic, pyrogenic, or biogenic). Traditionally, the fractionation of these compound classes was accomplished using column chromatography (i.e. silica gel, alumina, florisil), thin-layer chromatography (TLC), or high performance liquid chromatography (HPLC). Alzaga, et. al. (2004) first reported the use of a mixed bed SPE cartridge for the fast, quantitative separation of the two compound classes in fuel-oil samples. However, the method still requires hands-on manipulation of the fractionation and concentration processes which may affect precision and reproducibility.

The PrepLincTM SPEi system allows the user to control the conditioning, loading and elution of samples through commercially available cartridges and disks. With the addition of the AccuVap module, the elution and evaporation steps can be linked to obtain analysis ready samples contained in autosampler vials. In this study, the labor-intensive steps of fractionation and concentration were reduced to two steps: 1) loading samples on to the instrument and 2) programming the sequence with saved method parameters. This provided for a fast and simple method for the separation of aliphatic and aromatic components from petroleum related products and petroleum contaminated environmental samples.

Keywords: Automation, PAH, Petrochemical, Sample Preparation
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Carbon paste electrode (CPE) belongs to promising electrochemical sensors of wide applicability because of its several advantages such as low background currents, reproducible results, easy fabrication and modification with wide range of compounds. A distinct advantage of carbon paste-based electrodes is their very low ohmic resistance. Hence, the carbon paste electrode has been widely modified with other chemicals. In this study carbon paste electrode mixed with sericite (S-CPE) or sericite modified with hexadecyltrimethyl-ammonium bromide(MS-CPE) was used as a working electrode in cyclic voltammetry (CV) and measured arsenic (As(III) and As(V)) at different conditions such as concentration of arsenic, pH and ionic strength. It was observed that the performance of the resulting MS-CPE possessed significantly high signals in terms of cathodic or anodic current comparing to the CPE and S-CPE. The MS-CPE has been successfully employed for the detection of arsenic in the linear range of 2 - 50 μM with the detection limit of 1.0 μM in synthetic wastewater.
Development of Electron Tracking Compton Camera for Fine Imaging and Quantitatively Dose-Monitoring to Environmental Gamma-Ray

A new type of Compton camera, Electron Tracking Compton Camera (ETCC), have been extensively developed for imaging and monitoring environmental gamma-ray from contaminated soils. The decontamination of long-lived caesium (Cs) in soils below the regulation level of 0.23 uSv/h, is an issue of great concern at present in Japan. In the decontamination, fine and quantitative imaging capability is required for checking decontamination performance in contaminated areas. The ETCC realized fine imaging to the area by detecting a recoiled electron in the Compton scattering process using a gas (Ar/Ethane) scatterer. Owing to its precise trace capability of incident gamma-rays, the number of incoming gamma-rays can be directly associated with contrast of the reconstructed source image. This means the ETCC has potentials for not only an imaging device but an absolute radiation monitor in a field of view. Furthermore, background particles can be rejected by their energy-deposit (dE/dx) and track length differences in the gas. This enables to measure a weakly contaminated area around 0.1 uSv/h by improving a signal-to-noise ratio. We carried out performance tests with a prototype ETCC in the contaminated area and proved imaging capability. In the presentation, we will introduce the imaging device, show results of these performance tests, and explain a novel evaluation method to translate Cs distribution to the radiation dose.

Keywords: Environmental, Spectrometer
Application Code: Environmental
Methodology Code: Other
Environmental Samples - Sampling, Detection and Water/Soil Samples

Determination of Veterinary Antibiotics Found Within the Soil and Groundwater of Farmlands

In rural areas, livestock from farmlands are causing negative effects on the environment, and potentially human beings. Farmers pump their livestock with many different classes of antibiotics to keep them healthy and improve feed efficiency. Many of these antibiotics are excreted from the livestock still in their active forms. The surrounding area, including the water supply, can become contaminated with these active antibiotics. Identification of the antibiotics and their concentrations in the environment is important in understanding how extreme the negative effects are.

Extraction methods from solid and liquid samples, as well as identification of the antibiotics present in the environment will be presented. These proposed methods will be used to determine concentration of the antibiotics present in a nearby farmlands and their surrounding water supplies using HPLC.

Abstract Text

Keywords: Environmental/Soils, Environmental/Water, Liquid Chromatography, Pharmaceutical
Application Code: Environmental
Methodology Code: Liquid Chromatography
Non-renewable energy sources have been widely studied in recent years, though they tend to run out and cause major environmental impacts such as global warming. In this scenario, the search for renewable energy sources is a necessary alternative. The biomass potential renewable energy sources are becoming attractive alternative. The waste generated in the process of wastewater treatment is also seen as good option for energy reuse, driven by the National Solid Waste Policy, which highlights its energy recovery. The city of Araraquara / SP stands out on the national scene by having a modern system of dewatering and thermal drying of sewage sludge. In this study the residue from the Sewage Treatment Station of Araraquara was characterized after dewatering and thermal drying process, and conducting biological H2 production with anaerobic sludge from the sedimentation ponds tests. The results obtained with these residues shown high organic matter content, calorific value and generation of H2, opening new possibilities for their application as potential to generate clean, renewable energy.

Keywords: Biofuels, Energy, Environmental/Waste/Sludge, Thermal Analysis

Application Code: Environmental
Methodology Code: Thermal Analysis
Environmental Samples - Sampling, Detection and Water/Soil Samples

Development of Test Paper for Fluoride Ion with ON-OFF Color Change Reaction

Contamination of fluoride ion in drinking water has caused serious health problem such as mottled teeth in developing country. Therefore, simple fluoride analysis has been desired. As one of the effective on-site analyses, colorimetry has been used, because it is unnecessary analytical instrument for judgment of sample concentration. However, this method causes individual difference of analytical result, because of judge of concentration by color contrast. Recently, we have found the binary color reaction (ON-OFF color reaction) when aluminium ion mixed with Lanthanum alizarin complexone (La-ALC) which is color reagent for fluoride. Moreover, number of discoloring is changed with fluoride concentration using this binary color reaction. Based on these findings, we have already reported visual analysis for fluoride by counting number of discoloring using this binary color reaction. However, this method has a problem in operability and portability because this method is required solution reagent. In this study, we attempted to development of fluoride analysis paper with ON-OFF color reaction.

Abstract Text

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Keywords: Chromatography, Environmental Analysis, Environmental/Water, Method Development

Application Code: Environmental

Methodology Code: Other
Analyzing Total Organic Carbon in Sea Water

The analysis of Total Organic Carbon (TOC) in seawater can be both challenging and expensive. The concentration of organic carbon in sea water is of considerable interest. The effect this matrix can have on TOC analyzers can lead to rapid consumable turnover, costly maintenance and repairs. Laboratories are continually looking to increase sample throughput, decrease overall cost of analysis, and improve ease of use while maintaining reproducibility. This poster will demonstrate the ability to maximize throughput of seawater samples using a high temperature combustion analyzer for TOC analysis.

Keywords: Environmental Analysis, Environmental/Water, Total Organic Carbon, Water

Application Code: Environmental
Methodology Code: Other
Abstract Text

Phenols are widely used in various domestic and industrial applications; they are often released into the aquatic systems through wastewater discharges. Phenols have been reported to have harmful effects on man and the environment, hence the need to ensure their removal during wastewater treatment processes. This study assessed the potential of activated grape leaf litter (AGL) for the removal of 2-nitrophenol (2-NP) and 4-nitrophenol (4-NP) from wastewater. Batch adsorption studies were carried out on simulated wastewater. Adsorbent concentration, pH, contact time and temperature were optimised to achieve equilibrium adsorption conditions. Langmuir, Freundlich, Temkin and Dubinin-Radushkevich isotherm models were used to describe the adsorption process. Thermodynamic parameters were also evaluated. The optimal adsorption pH was 8 for the sorption of 2-NP and 4-NP. A further increase in initial pH resulted in decreased adsorption capacity. The adsorption equilibrium of 2-NP and 4-NP onto AGL was attained in 30 min. The adsorption kinetics for the sorption of 2-NP and 4-NP is best described by pseudo first order model (R² > 0.999). Freundlich isotherm best described the adsorption process. Thermodynamics studies revealed that removal processes were exothermic and that contaminant uptake was unfavourable at high temperatures. The optimum capacity of AGL for removal of 2-NP and 4-NP were 103.10 mg/g and 102.09 mg/g respectively. This study suggests that activated grape leaf litter may be utilized for the removal of nitrophenols from wastewaters in industrial processes.

Keywords: Adsorption, Contamination, Environmental/Water, HPLC
Application Code: Environmental
Methodology Code: Liquid Chromatography
In this study, we aimed to investigate the removal and decolorization of dye bearing textile effluents by urea-formaldehyde resins admixed with various proportions of modified Chitosan, Starch, eggshell, powder, rice husk, etc. Different textile effluents were collected and treated with these various mixtures. The resultant liquids were almost colorless with very low COD. The sludge can also be used as filler for plastic manufacturing, road paving or brick making. The produced adsorbent mixture may entity in environmental science. The adsorption properties of these adsorbents were investigated under different adsorption conditions with different initial dye concentrations, contact times and pH values. The adsorption equilibrium data were analyzed with Langmuir and Freundlich models. The adsorption behaviors of various textile effluents onto the resin mixtures were better described by the Freundlich model.

Keywords: Adsorption, Environmental Analysis, Environmental/Biological Samples, Environmental/Waste/Sludg
Application Code: Environmental
Methodology Code: Chemical Methods
The Application of Infrared Microspectroscopy for the Analysis of Microplastics in Water-Borne Environmental Samples

Since the 1950s mankind has produced billions of tonnes of polymers, with the rate of production increasing significantly on an annual basis. Handling of the disposal of used materials is becoming a major problem and increasing amounts are finding their way into the river system, coastal waters and eventually into the World’s oceans. The majority of plastic materials will degrade slowly. This process of degradation due to wave, wind and UV light can generate small particulates, called microplastics that can accumulate and, through digestion, enter the food chain.

In order to combat and identify the source of the pollution, identification of the bulk and microplastics is extremely important. Infrared spectroscopy is the primary analytical technique for the identification of polymers. If the samples of the plastics are greater than 50 microns in size then they can be analysed using an FTIR instrument equipped with an Attenuated Total Reflectance (ATR) sampling accessory. For microplastics, where the particles are below 50 microns in size, an infrared microscope or infrared imaging system can be deployed for the rapid measurement and identification of the plastic types. The microplastics for the infrared microspectroscopy were collected on filter membranes.

This study shows the application of ATR spectroscopy and infrared microscopy for the measurement of plastics and microplastics collected from European waterways from the river source to the river estuary.

Keywords: Environmental/Water, FTIR, Microspectroscopy, Polymers & Plastics
Application Code: Environmental
Methodology Code: Vibrational Spectroscopy
Environmental Samples - Sampling, Detection and Water/Soil Samples

Light Weight Evaporation Vessel for the Determination of Dissolved Solids

The determination of dissolved solids has long been a key parameter for water quality measurements. Water having high dissolved solids content could be unsuitable for use in industry. Water with concentrations greater than 500 mg/L usually ranks very low on a taste scale and can produce particularly adverse reactions amongst those who are not native to that water source. The test generally consists of evaporation in a tared dish of a known filtered volume of the water with subsequent weighing of the residue. These dishes need to be stable at the temperatures used as well as nonreactive to the matrix of the sample. Traditionally these dishes have been made of borosilicate glass, porcelain, or platinum. The difficulty often presented by these traditional materials is the weight difference between the container and the mass of residue produced. Most methods limit the mass of residue to be between 2.5 and 200 mg. They further require that the measured mass be reproducible to ± 0.5 mg. With such a large percentage of the mass being contributed by the dish the analyst is often confronted by unnecessarily long and drawn out testing sequences. The development of a lighter weight evaporation container will be discussed.

Keywords: Environmental, Environmental/Water
Application Code: Environmental
Methodology Code: Other
The current Contract Laboratory Program (CLP Method SOM01.1) and Environmental Protection Agency (EPA Method 3640A) Gel Permeation Chromatography cleanup procedures utilize 70 gram S-X3 Biobead columns, at a flow rate of 5mL/minute with dichloromethane as the mobile phase. The maximum lipid load on these traditional columns is 1 gram in a 5mL injection. With a run time of 1 hour, to carry out the separation of co-extracted, higher molecular weight interferences from the analytes of interest, the methods require 300mL of dichloromethane per sample injection. Both methods have between-peak resolution (85% and 90%), and analyte recovery range requirements.

The PrepLinc GPC-MAXX feature allows the user to control the GPC dump, collect and wash operations through 2 columns connected in-line. By allowing the guard column dump fraction to act as a molecular filter, the amount of material transferred to the second separation column is minimized which allows for more material to be loaded on a dual column system. Comparisons between the J2 GPC-Guard/Express column configuration and the traditional 70g columns will demonstrate a 1/3 savings in chlorinated solvent usage and run time. This study will also demonstrate the effectiveness of the GPC-MAXX feature by analyzing several different types of sediment and tissue extractions, with comparisons between analyses from traditional GPC Cleanup techniques and alternative column technologies. Finally, the usefulness of alternative column packing materials, to further increase co-extracted interference removal while decreasing overall runtime and chlorinated solvent usage, will also be demonstrated. In all cases, the peak resolution and recovery requirements were maintained to meet both CLP and EPA method requirements.
**Session Title**  
Environmental Samples - Sampling, Detection and Water/Soil Samples

**Abstract Title**  
Advanced Portable Water Quality Testing

**Primary Author**  
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**Abstract Text**

In this Abstract we will discuss the different methods for on site water quality testing. Items covered will be the different types of chemistry used and their introduction methods, known interferences, typical applications and data management. The purpose of this abstract will be to better understand the options available to professionals in multiple industries when it comes to water quality sampling and testing, along with any known issues that may arise, thus giving the attending professional a good idea of the correct testing methods needed for his or her specific application.

**Keywords:** Instrumentation, Laboratory, Portable Instruments, Spectrophotometry

**Application Code:** Other

**Methodology Code:** Portable Instruments
Development of 4-Channel LED-Based Reflective Photometer for Determination of Trace Elements in River Water Samples with Membrane Filter Extraction

For sensitive determination of various substances with easy operation, a reflective photometer using light-emitting diodes (LEDs) was developed. LEDs wavelengths were 430, 525, 635, and 740 nm, respectively, and they were turned on and off sequentially. Fiber optics was used to merge the LEDs emission and to illuminate the surface of a filter (sample), and another was used to collect the light reflected by the filter. The reflected light was detected by a photodiode (PD) and its intensity was acquired by a controller, synchronized with blinking of LEDs. Reflectance ([R]/[I]) against blank was calculated for each LED and it was converted to reflective absorbance ([log_{10}[R]]) and Kubelka-Munk function \((1 - [R])^2 / 2[R]\). Copper(II) was determined with bathocuproine chelating reagent. The chelate was collected on a filter and its reflective absorbance was measured at 525 nm. A calibration curve was linear between 0 and 0.1 mg/L and detection limit (DL, 3 of blank, [n] = 5) was 5 [micro]g/L. Recoveries for 1 [micro]g of copper added to 10-mL river water samples were measured and they were from 98 to 102%. Another application was phosphorous as phosphate determination by molybdenum blue method. A calibration curve (740 nm) was linear between 0 and 7.5 [micro]g/L and DL was 0.2 [micro]g/L. River water samples were analyzed by this method and official analytical method. Their results were in good agreement within 10% errors. Thus, the performance of the photometer could be successfully demonstrated.

This work was supported by JSPS KAKENHI Grand Number 24550098.

Keywords: Environmental/Water, Instrumentation, Solid Phase Extraction, UV-VIS Absorbance/Luminescence
Application Code: Environmental
Methodology Code: Portable Instruments
We present a new design for a portable paper-based sensing device for the colorimetric detection of Bisphenol A (BPA) in household dust. A rapid procedure for dust collection is used with a sensitive method for BPA detection, based on the formation of a greenish color on the test zone of the sensing device. The color results from the formation of a Schiff base compound, quinine-imine, formed by reaction of chitosan with the enzymatic product of tyrosinase, o-quinone, on paper coated in a layer-by-layer (LbL) assembly approach. The designed system includes a paper-based sensor disk with a diameter of 0.6 cm as a test zone for BPA detection, and the air-sampling cassette with a diameter of 37 mm as a collection area for household dust. A color database based on sRGB color space was designed specifically for BPA detection in household dust. Color intensity was evaluated using a statistical histogram provided by Photoshop. Colorimetric response was concentration dependent with a detection limit of 0.28 µg/g. The color started to appear within the first 60 s and stabilized after 30 min. Data from Gas Chromatography (GC) was validated our colorimetric data. Real household dust specimens were collected from four different homes and a day care center in Potsdam, NY, USA. Results showed a linear regression (R^2 = 0.9743) for real samples measured by both of the calorimetric and GC methods. In this work, BPA ranged in concentration from 0.05 to 3.87 µg/g in 57 samples of dust when both methods were used.
**Environmental Samples - Sampling, Detection and Water/Soil Samples**

**A Field Emission Ion Source for High Pressure Mass Spectrometry**

There is a growing demand for in-field analysis for many applications from environmental monitoring to defense. Handheld mass spectrometry brings a powerful tool to bear on these challenges and has been enabled through the development of high pressure mass spectrometry (HPMS). Operating miniature cylindrical ion traps (r[sub]0[/sub] < 1 mm) at elevated RF drive frequencies (f > 6 MHz) and pressure (P > 1 Torr), yields conditions where a turbomolecular pump is not required. In our lab, HPMS has been demonstrated with a variety of ionization sources including thermionic filaments, glow discharge, and electrospray ionization. However, there remains the need for a miniaturized ionization source that reduces size and power relative to previously developed sources.

The microionizer is a planar, silicon-based field emission source developed for minimal size, weight, and power, as well as compatibility with HPMS conditions. It has dimensions of 1 cm[sup]2[/sup] x 500 μm thick; decreasing volume by 90% relative to a glow discharge source. Field emission is controlled with voltage pulses between 10 to 100 V, generating electric fields on the order of 0.1 to 1 MV/cm with less than 10 mW power draw. Emission currents have been studied under HPMS conditions at 1 Torr of ambient air to confirm Fowler-Nordheim field emission. The current density was also shown to be sufficient for HPMS by collecting mass spectra of volatile organic compounds.

Funding for this project is provided by the Defense Threat Reduction Agency-Joint Science and Technology Office for Chemical and Biological Defense.

**Co-Authors:** J Michael Ramsey, Kenion H. Blakeman, Stanley Pau, Tina E. Stacy

**Abstract Text**

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**Keywords:** Ion Trap, Mass Spectrometry, Portable Instruments, Semiconductor

**Application Code:** Environmental

**Methodology Code:** Mass Spectrometry
Microfluidic paper-based devices (PBDs) have received significant attention for biochemical and medical diagnostics, but the applications to environmental testing are also significant, particularly for use in developing countries. This poster presents a new paper-based microfluidic device to measure the cadmium in water samples by titration with using colorimetric reagents. The reagents, based on a sulfonated naphthoquinone with a thiosemicarbazone moiety, react stoichiometrically within the wax-printed channels giving a color change whose intensity and area correspond to the cadmium concentration. We demonstrate that measuring both parameters can improve the calibration model and increase the sensitivity.

Keywords: Environmental/Water, Portable Instruments
Application Code: Environmental
Methodology Code: Microfluidics/Lab-on-a-Chip
We report on the thickness degradation of deposited silicon nitride layer, mainly used as passivation layer in the semiconductor manufacturing process, caused by airborne molecular contamination inside front opening unified pod (FOUP). An artificial contamination inside FOUP by airborne molecules was introduced to investigate the influence of contamination on the wafer surface. The concentration of fluoride ions artificially contaminated inside FOUP was 6,420 ppbv which was 400 times higher than that of a clean FOUP. The thickness degradation of the silicon nitride layer did not occur when bare wafers had been stored in a clean FOUP before the silicon nitride deposition process. Otherwise, the rate of thickness degradation of the silicon nitride layer gradually increased in the range of 6 Å to 11 Å according to the degree of decreasing layer thickness when the bare wafer had been stored in a contaminated FOUP. It appeared that some of remaining fluoride ions on the wafer surface that contaminated the wafers during etching process diffused in FOUP when the wafers are transported and stored. Then, the residual fluoride ions in FOUP migrated to the bare surfaces of the other clean wafers. These migrated fluoride ions could inhibit the formation of a silicon nitride layer. From this study, the cleaning and monitoring processes are essential and should be applied not only to the processed wafers but also to FOUPs after each chemical etching step.

Keywords: Contamination, Environmental Analysis, Monitoring, Semiconductor
Application Code: Environmental
Methodology Code: Other
High-resolution oxygen and hydrogen stable isotopes of surface snow and snow pits at the Greenland Environmental Observatory, Summit (GEOSummit), were measured by an Off Axis Integrated-Cavity Output Spectrometer (Los Gatos Research DLT-100). The year round, high temporal $^{18}\text{O}$ and $^{2}D$ in bi-weekly sampled surface snow during 2012-2014 showed clear seasonal variations. Enriched $^{18}\text{O}$ and $^{2}D$ values were observed in summer, while depleted values were measured in winter and spring samples. The greatest $^{18}\text{O}$ and $^{2}D$ variability was observed in the 4th quarter, whereas the 1st quarter showed less variability. $^{18}\text{O}$ and $^{2}D$ co-varied with air temperature when the most positive $^{18}\text{O}$ and $^{2}D$ values in late July 2012 corresponded to unusually warm weather caused by snow melting resulting in post-depositional modification of isotopes. Since water isotope fluctuations are governed primarily by atmospheric conditions, i.e. air temperature and associated air-mass trajectories, long term monitoring of interannual oscillation and magnitude provides insight into source, depositional, and post-depositional processes associated with hydrologic cycles and climate changes in the region. The $^{18}\text{O}$ and $^{2}D$ relationship in surface snow shows a slope similar to the global meteoric water line, at 7.97, compared to 8.0. Overall, $^{18}\text{O}$ and $^{2}D$ are well preserved in snow pits, with no to minimal post-depositional modification at GEOSummit, as $^{18}\text{O}$ and $^{2}D$ patterns lined up well over monthly snow pits. That is, isotope signals from surface layers corresponded to deeper layers in the proceeding months’ pits, except a few centimeters’ shift in depth. This offset matched well with the presumed weekly snow accumulation rates in different seasons (Banta et al. 2009). Our data support the previous assumption that no significant post-depositional processes occur in the high latitude high elevation of GeoSummit.

Keywords: Environmental Analysis, Isotope Ratio MS, Laser, Quality
Application Code: Environmental
Methodology Code: Other
A simple, novel electrochemical method for the detection of arsenic has been developed. The optimized method employs square wave anodic stripping voltammetry (SWASV) and does not require complicated or expensive electrode fabrication, as it utilizes a bare glassy carbon electrode. The co-deposition of copper greatly increases the sensitivity of the electrode allowing for detection limits in the low parts per billion. The method has successfully been used to detect arsenic in various aqueous samples that demonstrate the significance of this technique in environmental and industrial settings.
Great Lake Coastal Wetlands are essential ecosystems. They provide a variety of services including water quality control, wildlife habitat, flood, pollution, and erosion control, and carbon sequestration. They are continuously storing and emitting greenhouse gases (GHG) such as Methane (CH4), Carbon Dioxide (CO2), and Nitrous Oxide (N2O). My project is monitoring how the GHG flux between the wetland atmosphere interface changes when the wetland experiences drying.
In the environment, trace metals are aggressive environmental pollutants due to their rapid mobilization and transportation. The bio-availability and toxicity of these metals are controlled by their complexation with different ligands. Most environmental analysis techniques assume the equilibrium of these complexation processes, but these reactions are often not at equilibrium. A real-time detection technique which measures free metals is necessary to characterize dynamic metal complexation. Traditional analytical methods are very sensitive but do not measure in real time. We developed a real time detection technique to characterize metal complexation using Fast scan cyclic voltammetry (FSCV). Due to its well established thermodynamic complexation properties; we used Cu (II) complexation with different known ligands to verify our paradigm. We developed a hydrodynamic model to explain the complexation and compared it with a thermodynamic model for further verification. The ultimate goal of our project is to use our FSCV method as an [i]in situ[/i] real time diagnostic tool for metal toxicity in the environment.

Keywords: Electrochemistry, Environmental Analysis
Application Code: Environmental
Methodology Code: Electrochemistry
Trace metals are highly toxic pollutants in environment and have caused major concerns to public health. Our earlier work found Fast-Scan Cyclic Voltammetry (FSCV) coupled with carbon fiber microelectrodes (CFMs) particularly powerful in real-time detection of Cu and Pb in aqueous environmental samples. Now we functionalize CFMs to improve selectivity by a covalent scaffolding strategy. We employ two schemes in our modification: the first one is creating scaffolds on CFMs via electrochemical reduction of silylated ethynylaryl diazonium salts, making the density of the scaffolds dependent on the silyl spacers. The second scheme is attaching ionophores to the desilylated scaffolds via Copper Catalyzed Azide-Alkyne Cycloaddition (CuAAC), rendering CFMs selective to copper. Moreover, we optimize the parameters for the two schemes and conclude a modular process to graft a variety of functional azides onto CFMs. We evaluate the modified electrodes’ sensitivity, selectivity and stability. Our covalent functionalization strategy for CFMs makes FSCV extremely effective for real time environmental trace metal detection with high selectivity.


Keywords: Chemically Modified Electrodes, Electrochemistry, Environmental, Microelectrode

Application Code: Environmental
Methodology Code: Electrochemistry
The color is a physical characteristic in the water produced by organic and inorganic substances found in a solution or colloidal particles. In natural waters, color is generally associated with metallic salts and organic substances; in this respect the color varies from yellow to brown while in wastewaters color is considerate as an associated pollutant with industrial processes. The currently method for measuring natural water color is the Hanzen method, because it produces colors, similar to those found in natural waters. When the water’s color is produced from industrial waste, the test of color with the platinum-cobalt method does not prove applicable because the hue does not fit the yellow-brown scale. Because of the previously mentioned issue, this investigation was focused on testing the ADMI method as an alternative of color measuring in industrial discharges in Medellin (Colombia).

As a result, a correlation was established among ADMI, pH, turbidity, conductivity, suspended solids, total solids, COD (chemistry oxygen demand) and BOD (biological oxygen demand) taken from 526 samples of wastewaters from ten industrial sectors that flow into The Medellin river. It is important to highlight that during this investigation none of the initial conditions of the analyzed samples were modified, so that the typical characteristics of color could be tested in every one of the industrial sectors that were tested.

Keywords: Characterization, Environmental/Water, Process Analytical Chemistry, UV-VIS Absorbance/Luminescence
Application Code: Environmental
Methodology Code: Process Analytical Techniques
Endocrine disrupters (EDs) are compounds that specifically interfere with the normal functions of the body’s endocrine system (system of glands for hormonal secretion) by causing adverse effects in both human and wildlife. Therefore, the routine, sensitive, reliable and cost effective detection methods for EDs are highly demanded.

In this work we present the selection and characterization of ssDNA aptamers that exhibit high affinity, specificity and sensitivity to progesterone (P4), 17b-estradiol (E2) and, norethisterone (NET). The aptamer selection was conducted by in vitro selections by incubating a huge ssDNA library of about 1.80x(10)^15 random sequences with the target analyte which has been previously immobilized on agarose beads. After certain cycles of selections, the enriched aptamers pool was cloned and sequenced. The dissociation constants (KD) of the selected aptamers were determined by fluorometry and by electrochemical impedance spectroscopy (EIS) methods. The KD values are in the low nanomolar range. Cross-reactivity tests for each aptamer demonstrated the high specificity to their target analyte. Additionally, circular dichroism (CD) spectroscopy measurements of the selected aptamers showed a remarkable conformational change upon binding the target analyte. The change in the conformation was exploited to design a biosensing platform to get a measurable signal after binding the specific analyte by following the redox process of a couple composed for [Fe(CN)6]3-/4-. For first time, an impedimetric aptasensor to detect progesterone was developed by yielding a limit of detection of 0.90ng/mL; for other analytes, the data will be presented and discussed in the programmed session of the conference.

We believe that the continuous selection of high affinity aptamers for other EDs and their integration in a biosensing platform will facilitate the routine monitoring of EDs in environment as well as clinical and medical diagnosis purposes.
Highly selective and sensitive phosphate sensors have been fabricated by constructing a solid membrane disk consisting of variable mixtures of aluminum powder (Al), aluminum phosphate (AlPO$_4$) and powdered copper (Cu). Both binary and ternary electrode systems were produced. The ternary membranes exhibit greater selectivity over a wide range of concentrations. The ternary electrode with the composition 25% AlPO$_4$, 25% Cu and 50% Al was selected as our preferred electrode. The ternary membrane electrodes exhibited linear potential response in the concentration range of $1.0 \times 10^{-1}$ to $1.0 \times 10^{-6}$ mol L$^{-1}$. The mechanism for the selectivity of phosphates by the electrodes includes adsorption, absorption and ion exchange processes. An understanding of these processes reveals that the composition of the membrane material and its molecular structural framework are all important. The layered double hydroxides which form within the interstitial layers of the phosphate selective membranes contribute to the selectivity of the ions.

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Keywords: Adsorption, Electrode Surfaces, Environmental, Ion Selective Electrodes
Application Code: Environmental
Methodology Code: Sensors
Chromium is a naturally occurring element present in water, rocks, soils, and in biological organisms. Chromium exists in various oxidation states, however, the only biologically and environmentally stable forms are trivalent Cr(III) and hexavalent Cr(VI) chromium. While Cr(III) is an essential nutrient for metabolism, Cr(VI) is toxic and a known carcinogen. Inhalation or ingestion of contaminated air, water, or soil, by Cr(VI) is a major concern for industries using chromate painting, electroplating and welding. Therefore, monitoring Cr(VI) levels to protect workers at various working environment is a priority. Filters commonly used for sampling in industrial hygiene all have some limitations, in particularly, stabilizing Cr(VI) for a period of time before analysis. At High-Purity Standards we are investigating how to extend the stability of low levels of Cr(VI) on various filter media. A variety of source materials for Cr(VI) are used to deposit on different filter media. Various conditions are tested for pre-treatment of filters before use, preparation of Cr(VI) on filter, digestion methods, and appropriate packaging and storage. Total chromium, and speciation of Cr(III) and Cr(VI) are determined by analytical techniques with comparison and spiking methods using proper instrumentations. The evaluation of the effectiveness on the stability of Cr(VI) filter associated with analytical results, precision, and bias will be discussed.

**Abstract Text**

Chromium is a naturally occurring element present in water, rocks, soils, and in biological organisms. Chromium exists in various oxidation states, however, the only biologically and environmentally stable forms are trivalent Cr(III) and hexavalent Cr(VI) chromium. While Cr(III) is an essential nutrient for metabolism, Cr(VI) is toxic and a known carcinogen. Inhalation or ingestion of contaminated air, water, or soil, by Cr(VI) is a major concern for industries using chromate painting, electroplating and welding. Therefore, monitoring Cr(VI) levels to protect workers at various working environment is a priority. Filters commonly used for sampling in industrial hygiene all have some limitations, in particularly, stabilizing Cr(VI) for a period of time before analysis. At High-Purity Standards we are investigating how to extend the stability of low levels of Cr(VI) on various filter media. A variety of source materials for Cr(VI) are used to deposit on different filter media. Various conditions are tested for pre-treatment of filters before use, preparation of Cr(VI) on filter, digestion methods, and appropriate packaging and storage. Total chromium, and speciation of Cr(III) and Cr(VI) are determined by analytical techniques with comparison and spiking methods using proper instrumentations. The evaluation of the effectiveness on the stability of Cr(VI) filter associated with analytical results, precision, and bias will be discussed.

**Keywords:** Chemical, Environmental, ICP-MS, Industrial Hygiene

**Application Code:** Industrial Hygiene

**Methodology Code:** Mass Spectrometry
Smouldering remediation is capable of achieving 99.9+% destruction of coal tar and hydrocarbon contaminants in porous materials. These porous materials experience temperatures in the range of 500-1100°C for periods of minutes or hours depending on contaminant type and operating conditions. Often, contaminated materials contain potentially toxic element (PTE) co-contaminants such as mercury and arsenic. High temperatures and smouldering reactions affect physical and chemical properties of materials subjected to remediation, which, in turn, affect the presence and fate of PTEs in those materials.

The Leaching Environmental Assessment Framework (LEAF) provides a collection of tests, data management tools, and assessment approaches to develop source term release profiles for a wide range of materials. LEAF tests were recently added to the USEPA SW846 compendium as standard methods 1313-1316.

The two materials in this study were a field-obtained soil artificially contaminated with coal tar and a field-obtained made ground from a coal tar contaminated site in the USA. These materials were subjected to smouldering remediation in the laboratory. Mercury and arsenic fate and speciation were studied through the application of cold vapour and hydride generation atomic fluorescence spectroscopy (AFS) in conjunction with LEAF testing and geochemical speciation modelling using LeachXS. Total PTE content seemed to be reduced through volatilisation during remediation. After remediation, changes in soil composition and chemistry affected aqueous release of mercury and arsenic from these soils as well as speciation. This knowledge can be applied to the design of engineering approaches to reduce PTE contaminant levels or availability at contaminated sites.

**Keywords:** Atomic Spectroscopy, Environmental/Waste/Sludge, Mercury, Speciation

**Application Code:** Environmental

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
Several regulatory bodies including WHO and USEPA have listed Polychlorinated biphenyls (PCBs) and Organochlorine pesticides (OCPs) among the potentially toxic compounds. Some of the hazardous health risks in humans include reproductive failures, nervous system dysfunction and cancers. In current study, we evaluate the mean concentrations of PCBs and OCPs in plasma samples of several humans residence in Lagos as well as in water samples of Lagos lagoon, Nigeria employing various extraction techniques (solid and liquid phase extraction) and analysed with gas chromatography coupled to electron capture detector. Mean concentrations of PCBs and OCPs (ng/µl) in plasma samples are as follows: PCBs 28 (0.0432-1.3249); 52 (0.0039-0.1678); 101 (0.0064-0.0511); 137 (ND-0.0329); 153 (ND-0.0665); 180 (ND-0.0397); 194 (ND-0.0730); lindane (0.00286-0.0481), chlorothalonil (0.00805-0.0148), heptachlor (0.00224 - 0.00318), aldrin (0.00099 - 0.0821), BHC (0.000310-0.0168), heptachlor epoxide (0-0.0006), endosulfan (0.00105 - 0.1653); dieldrin (0.00104 - 0.00926); P,P'DDD (0.0128 - 0.9499); endrin (0.01198 - 0.02315); P,P'DDT (0.01501 - 0.06697); methoxychlor (0.01016-0.09181); lambda cyalothrin (0.04103-0.06402) and permethrin (0.05572-0.48793). Mean concentrations of PCB 28 and lindane were highest while those of PCB 180 and heptachlor epoxide were lowest. The values in plasma samples were below the maximum concentration limits (MCL) approved by European Union (EU) for organochlorines.. The concentration levels in this study were compared with the levels detected in other parts of the world. The study confirms PCBs and OCPs persistence in Lagos, despite its ban several decades ago.

Keywords: Chromatography, Environmental/Water, Extraction, GC Detectors
Application Code: Environmental
Methodology Code: Gas Chromatography
Environmental contamination has been at the forefront of government policy and regulation since the USEPA was established in 1970. Over the years the US EPA has developed, published, and updated multiple methods for analysis of environmental pollutants, and single-quadrupole gas chromatography-mass spectrometry (GC/MS) has long been the technique of choice for determination of volatile organic contaminants (VOCs). As efforts to provide dependable analytical methods has progressed, the GC/MS instrumentation has evolved, with improvements in sensitivity, reliability, and user experience, but there haven’t been any significant advancements in the overall methodology since the mid-1980s.

The USEPA is currently in the process of developing a revision to USEPA Method 624, which was first promulgated in 1984 and specified the use of packed columns as part of the protocol to collect data on the proposed pollutants. As part of the revision, the USEPA is evaluating up to 30 additional pollutants for inclusion in the method.

This poster will provide examples from a purge-and-trap method validation study conducted to evaluate operating conditions for the existing VOC list plus the proposed list of 30 new analytes. Column selection and GCMS instrumental recommendations will also be addressed to bring the method in line with modern methods. Calibration levels along with QC criteria will also be presented.

Keywords: Environmental, Gas Chromatography/Mass Spectrometry, Purge and Trap, Volatile Organic Compounds
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Environmental Samples - Separation Techniques

Parts-per-Trillion SVOC Air Analysis with Novel Optical GC Detector

Ambient air analysis is now performed widely in homes and small businesses as well as the more traditional industrial locations as the general public and businesses wish to better understand their air quality. These ambient air methods customarily look for VOCs, more volatile SVOCs, mold VOCs, and combustion gases like NOx, CO, and CO2. SVOCs are normally measured by collecting dust samples, which are subsequently extracted and/or prepared for analysis, instead of air samples because the concentration in ambient air is too low for traditional environmental analytical methods. Unfortunately, dust samples are generally not representative of the sample location due to the large area that needs to be evenly sampled, much of which may be inaccessible due to furniture or other structures. If an air sample could be collected and analyzed with appropriate sensitivity and selectivity, a more representative level of SVOCs in the home or business could be determined.

A novel optical GC detection system (MAX™) has been developed that provides both quantitative and qualitative determination of 100s of SVOCs at or below the single digit parts-per-trillion range (airborne concentration) from a single sample. The MAX™ detection system utilizes optical spectroscopy to provide a one-time permanent calibration so many compounds can be quantified in every sample. Increasingly costly helium is not required as a carrier gas, and the system can be setup and running very quickly since high vacuum is not required as in the more traditional GC-MS based analyses. The novel MAX™ detection technology and its application to SVOC air analysis will be discussed.

| Keywords: | Environmental/Air, Gas, Instrumentation, Spectroscopy |
| Application Code: | Environmental |
| Methodology Code: | Gas Chromatography |
Due to the high concentrations of inorganic ions (e.g., Cl-, Br-) and the complex sources of organic materials, the chemistry associated with disinfection of seawater is more complicated than that of fresh water. However, up to date little is known about the concentration levels, species distributions, and influential factors about the disinfection byproducts (DBPs) formed during aquarium seawater disinfection.

In this study, the effects of organic precursor, Br- and pre-ozonation upon chlorination/chloramination of Beijing Aquarium seawater on the formation and associated bromine substitution factor (BSF) values of several typical classes of DBPs, including trihalomethanes (THMs), haloacetic acids (HAAs), and haloacetamides (HAcAms) were investigated. The results indicated that, with an increased dissolved organic carbon concentration, the formation of THMs and HAAs increased by 3.2-7.8 times upon chlorination and by 1.1-2.3 times upon chloramination. An increase in the Br-concentration substantially increased the concentrations of THMs and HAcAms and the BSF values of all detected DBPs, while the total yield of HAAs changed slightly upon both chlorination and chloramination. Pre-ozonation treatment (1.0 mg L-1 ozone dose) obviously suppressed the formation of all studied DBPs during subsequent chlorination, but promoted to some extent the DBPs formation during subsequent chloramination. The BSF values of all studied DBPs increased after pre-ozonation treatment even at a low ozone dose.

In summary, for aquarium seawater disinfection, chloramination produces much less DBPs than chlorination. However, if chlorine is adopted as disinfectant, pre-ozonation can substantially reduce the formation of studied DBPs.

Keywords: Environmental Analysis, Gas Chromatography, Toxicology, Water
Application Code: Environmental
Methodology Code: Gas Chromatography
The development of a method to determine the amount of endocrine-disrupting chemicals (EDCs) in drinking water using Gas Chromatography- Mass Spectrometry (GC-MS) and Liquid Chromatography will be presented. EDCs can be harmful to aquatic life and humans in specific concentrations potentially causing developmental, reproductive and neurological effects. I aim to quantify the concentration of Estrone (E1), 17$\beta$-ethinylestradiol (EE2) and Estriol (E3) from water samples collected in Herkimer County, NY and further determine how to safely remove these chemicals from the water.

**Keywords:** Environmental/Water, Gas Chromatography/Mass Spectrometry, Liquid Chromatography

**Application Code:** Environmental

**Methodology Code:** Liquid Chromatography
The inertness of a GC column has prime importance in the analysis of active sample compounds. Active sites on a GC column or on a stationary phase interact with the sample components having acidic, basic or hydrogen bonding properties. Due to such interactions, compounds can be adsorbed on the active sites, which results in decomposition or peak tailing or reduction in peak response and non-reproducible results for run-to-run analysis. Consistency and accurate analysis of environmental and other samples consisting of basic, acidic and alcohol groups containing compounds is very important to assess the true toxicity of a particular sample. In the current investigation, two inert columns were developed and evaluated for the analysis of active components containing samples specified in USEPA Method 8270. Also, pesticide samples were analyzed on the inert columns and results were compared with standard GC columns. Lower detection limits, peak tailing, and linearity range for active and neutral components in a standard mixture were studied using inert and standard GC columns. The comparison of the data illustrates the importance of inertness of the column for the accurate determination of active groups containing compounds by GC and GC/MS.

**Keywords:** Capillary GC, Environmental Analysis, Gas Chromatography/Mass Spectrometry, GC Columns

**Application Code:** Environmental

**Methodology Code:** Gas Chromatography/Mass Spectrometry
### Session Title
Environmental Samples - Separation Techniques

### Abstract Title
Validation of Environmental Water Methods on One System: Considerations for Sample Volume, Purge Parameters and Quality Control Parameters

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#### Abstract Text
Water quality laboratories across the nation are faced with both a rising level of water quality awareness amongst the general public, as well as rising costs in water quality monitoring. As a result, laboratories are looking for more efficient ways to provide higher quality monitoring.

This study compares US EPA methods 524.2 and 524.3, as well as 8260B and 8260C, all on a single system. This study focuses on considerations for sample volume and purge parameters for the US EPA 524 methods, and considerations for quality control parameters for the US EPA 8260 methods.

#### Keywords
- Environmental Analysis
- Environmental/Water
- Gas Chromatography/Mass Spectrometry
- Purge and

#### Application Code
- Environmental

#### Methodology Code
- Gas Chromatography/Mass Spectrometry
Siloxane measurement in biogas samples has become important to prevent the destructive deposition of silicon dioxide on power generation systems during siloxane combustion. Filters are available for removing siloxanes in the biogas, but monitoring of the inlet and outlet feeds is necessary to verify that filter breakthrough is not occurring. Generally, filter breakthrough occurs slowly enough that occasional sampling and analysis is sufficient to reliably determine when filter replacement is needed.

Canisters provide one of the simplest collection techniques for monitoring air, as the contained vacuum simply pulls in more than enough sample needed to quantify levels of contaminants in the laboratory. Unfortunately, SUMMA passivated and other types of canisters used previously were not inert enough to store and recover the full range of siloxanes quantitatively. A new canister is presented that uses a more inert ceramic lining on the internal surface of the canister that allows the full range of siloxanes to be recovered up to 2 weeks after sampling. A laboratory preparation system automates the GCMS analysis of up to 20 canisters, with optional heating up to 150 deg C prior to sample extraction. Data for Siloxanes with a molecular weight out to L5 and D6 will be presented, including calibration curves, method detection limits, and holding time studies that demonstrate the effectiveness of these new sampling canisters and laboratory sample preparation systems.

Keywords: Air, Environmental, Gas Chromatography/Mass Spectrometry, Volatile Organic Compounds
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Isocyanates are a main component in the production of polyurethane (PUR) materials. Exposure to isocyanates can put workers at risk for respiratory disorders like “occupational asthma”. It is important to include monomer and oligomer isocyanate species when performing environmental health and safety exposure assessments as many paints, coatings and spray foams formulations contain multiple species of isocyanates. The measurement of isocyanate urea derivatives by LC-MS-MS with sub 2µm HPLC column can provide fast analysis with optimum resolution and low detection limits. Columns packed with high purity, totally porous, monodisperse silica particles were used in this study and allowed for improved UHPLC performance and higher efficiency separations. Isocyanate monomers and oligomers were measured in one single injection into the UHPLC C18 column packed with 1.9 µm size particles. The chromatographic results will be pictured and evaluated. The study was done for multiple isocyanates including HDI, HDI oligomers, 2,4-TDI, 2,6-TDI, MDI, MDI oligomers, IPDI, and IPDI oligomers.

Keywords: Air, Environmental, Industrial Hygiene, Paint/Coatings
Application Code: Environmental
Methodology Code: Other
Due to potential adverse health effects of certain chemicals used in consumer products, alternative chemicals are routinely introduced in the market. Understanding human exposure to these chemicals is necessary for risk assessment. Biomonitoring measures the body burden of chemicals through the quantification of the concentrations of the chemicals or their metabolites in biological matrices. Therefore, identification of metabolites which can be used as exposure biomarkers is important. In-vivo and in-vitro metabolism have been used routinely to identify metabolites during drug development. We used a similar approach to identify metabolites of emerging environmental chemicals to investigate the in-vivo metabolism of di-2-ethylhexyl tetrabromo phthalate, an emerging flame retardant, and cyclohexane di-isononyl ester, a phthalate alternative, in rats after administration of the chemical intravenously or by gavage. Similarly, we also investigated the in-vitro metabolism of di-2-ethylhexyl terephthalate and di-2-ethylhexyl-adipate using human liver microsomes. We monitored the differences in elution patterns of chemicals in the urine of dosed vs. control rats and the human liver microsome suspension with and without the parent chemical. We used mass spectrometric fragmentation patterns after HPLC separation to tentatively identify the in-vivo and in-vitro metabolites. Upon obtaining analytical standards of the metabolites that are specific to the chemicals studied, we are able to incorporate some of these newly identified biomarkers into ongoing biomonitoring programs such as the National Health and Nutrition Examination Survey.

**Keywords:** Environmental/Biological Samples, Identification, Mass Spectrometry, Metabolomics

**Application Code:** Environmental

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Environmental Samples - Separation Techniques

A Comparative Study of TOC and THM Analysis of Municipal Drinking Water Using Heated Sodium Persulfate Oxidation and a Novel Approach to EPA Method 524.4

Total organic carbon is an important indicator of water quality throughout the water treatment process. Raw source water is progressively treated to remove particulate matter and natural organic matter (NOM). Humic acid and fulvic acids contained in the residual NOM of water undergoing disinfection by chlorination react with chlorine to from disinfection by product compounds such as trihalomethanes (THMs) and haloacetic acids (HAAs), which have been linked to cancer. THMs continue to form during drinking water distribution due to excess chlorine levels required to maintain microbial disinfection.

TOC analysis is a quick and effective indicator of NOM and THM levels in source water and finished water. However, TOC analysis cannot be substituted for THM analysis and THM analysis requires significantly more time. The relationship between TOC from natural organic matter (NOM) in raw water, TOC levels reduced during treatment, TOC and THMs in finished drinking water, and TOC and THMs from points in the distribution system must be established in a timely manner for effective operation of the water treatment process.

This poster will present comparative data from TOC analysis employing the heated sodium persulfate oxidation technique in USEPA-approved method SM 5310C, and an improved approach to THM analysis using USEPA-approved method 524.4. Any relationship between TOC and THM concentration will be demonstrated and an understanding of the influence of chlorine dosing, time and other factors on each measurement parameter investigated.

Keywords: Environmental/Water, Gas Chromatography/Mass Spectrometry, Total Organic Carbon, Water Application Code: Environmental Methodology Code: Gas Chromatography/Mass Spectrometry
Since medical marijuana (MM) was legalized in California in 1996, 23 states and Washington, D.C. have passed laws allowing its use for a variety of medical conditions. From a consumer safety point-of-view, quantitation of the pesticide residues in MM products has begun to attract wide interest. There are several problems associated with analysis of pesticide residues in MM. First and foremost, there are very few regulatory guidelines established to define which pesticides to include or what the detection limits should be, and secondly the matrix is very complex with significant interferences. Finally, sample load is growing exponentially, so the chosen method must be quick and easy to perform. Trace level pesticide analysis in complex food matrices have been done for many years with similar challenges, thus many of the analytical protocols emerging for the MM matrix are based on these well-established techniques.

Gas Chromatography mass spectrometry (GCMS) is well established for trace level detection of pesticides in complex matrices. Triple-quadrupole GC-MS/MS operated in MRM mode provides significant improvements in both sensitivity and selectivity, but method development can be expensive and time consuming.

This poster describes streamlined screening of pesticide residues in MM using a QuEChERS sample preparation method, followed by analysis using a commercially available GC-MS/MS method package, which includes simultaneous Scan/MRM acquisition methods, second-column confirmation, and pre-registered calibration curves for semi-quantitative results for 478 pesticides. Tools for quick development of a fully quantitative Scan/MRM method for custom pesticide lists will be discussed in detail.

Keywords: Drugs, Forensics, Gas Chromatography/Mass Spectrometry, Pesticides
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Geochemical analysis of the <150 µm fraction of surface soils by neutron activation analysis and X-ray fluorescence spectrometry revealed that in general, strongly weathered soils overlying Miocene White Limestone Supergroup in large sections of Central Jamaica are characterized by unusually high levels of Cd. The source of the Cd remains controversial but is presumably natural (geological) since there are no sources of contamination in the area large enough to account for the widespread high levels. Here, we determined the Cd isotopic composition of soil samples, a cadmiferous phosphorite soil concretion, African dust and volcanic ash deposits (n = 21) to investigate the source of Cd in this region. The samples were appropriately digested and a portion of each digest double spiked ([sup]110[/sup]Cd–[sup]111[/sup]Cd) to correct for mass bias and experimental induced fractionation. Spiked samples were then purified by anion-exchange chromatography in order to pre-concentrate Cd in the samples, remove major elements and avoid isobaric interference before Cd isotopic determination using a multi-collector ICP-MS. The isotopic composition of the samples varied widely, with $[\frac{^{114}Cd}{^{110}Cd}]_{\text{sample}} = [\frac{^{114}Cd}{^{110}Cd}]_{\text{JMC Cd Münster}} - 1 \times 10^{-4}$ ranging from +0.2 to +9.0, suggesting that these soils do not solely reflect the underlying karstic geochemistry of the region. Although not precisely defined, there is also good evidence to suggest two distinct sources of Cd, which do not include the cadmiferous phosphorite soil concretion as previously suggested.

Acknowledgements: We wish to acknowledge the IAEA, University of the West Indies, Mona and the Ministry of Science, Technology, Energy and Mining, Jamaica for vital financial support.

Keywords: Environmental/Soils, ICP-MS, Metals, Nuclear Analytical Applications

Application Code: Environmental

Methodology Code: Mass Spectrometry
Electro-organic synthesis has received great interest as a new environmental compatible synthesis route to produce unique structures. Different monomers especially substituted aromatic compounds have been utilized to produce electrochemical conducting and non-conducting polymers. Functional polymers are attractive candidate for use as a chelating agents for the determination of heavy metals in water samples. [1-3]

We demonstrate here, the application of electro-reduction product of 4-nitrophenol coated on the activated surface the multi-wall carbon nanotubes (MWCNTs). The modified MWCNTS is successfully applied as dispersive micro solid phase extraction (DMSPE) to preconcentrate the Ni2+ from water prior to flame atomic absorption spectrometry (FAAS).

The 4-nitrophenol is reduced in a separated electrochemical cell in aqueous medium containing sodium acetate and NaCl on lead cathode and dimensional stable anode. The electro-reduction is conducted under constant current (17 mA). After electrochemical synthesis, a dark brown precipitated powder is washed by double-distilled water and subsequently dried. Furthermore, the product is characterized by analytical technique (GPC, DSC, TGA, FT-IR, 1H-NMR and cyclic voltammetry). After that, the product is coated on the surface of carbon nanotubes in water-methanol (1:1) medium. In the end, the dried electro-product modified carbon nanotubes is applied to determine the trace amount of Ni2+ by DMSPE-FAAS.

The recovery percentage and the detection of limit are obtained 98.7 % and 0.26 µg/L respectively. Finally, the method is employed for the determination of Ni2+ ions in some industrial fluids and petrochemicals waste waters.


Keywords: Environmental Analysis, Environmental/Water, Separation Sciences, Solid Phase Extraction
Application Code: Environmental
Methodology Code: Separation Sciences
One requirement found in all USEPA methods for volatile organic compounds (VOCs) is that specific GC/MS tuning criteria must be met before analyzing actual samples. The GC/MS is tuned using the traditional tuning compound, PFTBA (perfluorotributylamine), and the tune is evaluated every 12 hours by injecting BFB (4-bromofluorobenzene) and measuring the intensity of key mass fragments. The MS tuning procedures adjust PFTBA ion responses to achieve the desired BFB response ratios. The relative ion abundance of the BFB mass fragments must meet specific criteria established in the methods to ensure that the GC/MS instrument operating conditions are adjusted and optimized for analysis of VOCs, and the criteria must be met every 12 hours to ensure that the instrument performance remains stable enough for continued analysis.

The BFB tuning criteria for the three most common USEPA VOC methods (8260, 524, and 624) are very similar, although there are a few important differences. In addition, there are many factors that can affect the instrument’s ability to meet the specified criteria, including cleanliness of the ion source, GC oven temperature and column flow rate, threshold setting, and the tuning parameters themselves. This poster summarizes the differences in BFB tune requirements for the 3 most common USEPA VOC methods, and discusses the variables critical to meeting BFB tuning criteria. Recommended tuning conditions are included, as well as results from a long-term stability study.

**Keywords:** Environmental, GC-MS, Purge and Trap, Volatile Organic Compounds

**Application Code:** Environmental

**Methodology Code:** Gas Chromatography/Mass Spectrometry
It is well recognized that the textile industry is one of the world's largest users of potable water. Supercritical CO2 (scCO2) solvent characteristics can allow it to replace water in many textile operations such as scouring and dyeing, thereby significantly reducing or eliminating water demand.

Having built our first commercial scCO2 textile dye system in the 1997, we are again focused on further development and scaling up the use of scCO2 as a solvent in the textile industry.

This paper will focus on the application of scCO2 to the following textile operations and the accompanied reduction in water, energy and chemicals use and waste generated.

Scouring: Nylon and cotton fabrics were scoured using with scCO2 and showed extraction efficiencies > 95%. Scouring of cotton with scCO2 was also comparable to better than conventional water based scouring. The scCO2 scoured fabrics were tested for mechanical properties and for dyeing by conventional means and were found to be acceptable.

Dyeing of fabric and garments: Polyester and nylon yarn, fabric and garments were dyed in scCO2 using raw or non-formulated disperse dyes, and mixtures of raw dyes. The color uptake in the fabric was measured using a Datacolor colorimeter. A novel dye vessel design and process cycle have been developed which allows reproducible and uniform dyeing in a < 2 hour cycle. Commercial scale-up continues.

Dye Recovery: We have demonstrated in a potential new application in which a significant portion of a dye or dye mixture infused into polyester or nylon can be recovered for re-use by extracting with scCO2. This is not possible in the dyeing process practiced today.

**Keywords:** Consumer Products, Environmental/Water, Instrumentation, SFE
**Application Code:** Consumer Products
**Methodology Code:** Separation Sciences
A novel thiourea-based sorbent was prepared from N-{2-[Bis(2-aminoethyl)amino]ethyl}aminomethyl–polystyrene resin by chemical modification of resin with 3-pyridyl isothiocyanate. The new sorbent was characterized with FT-IR and examined for solid phase extraction of noble metals, including Au(III), Ir(III), Pd(II), Pt(II) and Ru(III). Sorption efficiency was studied via both off-line and on-line solid phase extraction (SPE). ICP-MS analysis of the aqueous solutions indicated that the sorbent possessed high selectivity allowing quantitative sorption (93–99%) of Au(III), Ir(III), Pd(II), Pt(II) and Ru(III) from relatively acidic solutions (pH: 1.0-2.0). The sorption of common transition metals, such as Cu, Co, Ni, Fe, Mn and Zn was insignificant. The analytes retained on the sorbent at pH 2.0 were eluted with 0.05 M thiourea in 0.1 M HCl and measured by ICP-MS. The procedure was successfully applied for SPE determination of Au, Ir, Pd, Pt and Ru in geological and soil samples by ICP-MS.

**Keywords:** Environmental Analysis, ICP-MS, SPME

**Application Code:** Environmental

**Methodology Code:** Separation Sciences
A new chelating resin of N-{2-[Bis(2-aminoethyl)amino]ethyl}aminomethyl–polystyrene polymer was prepared by anchoring salicylaldehyde onto the resin via -C=N- group. The salicylaldehyde functionalized resin was characterized by FT-IR, and then used as packing for separation of uranium(VI) and thorium(IV) in seawater and sludge samples by means of solid phase extraction (SPE) approach for ICP-MS determination. The influences of various analytical parameters, including load pH, eluent concentration, sample and eluent flow rates have been investigated on the sorption of U(VI) and Th(IV) on the chelating polymer. The U(VI) and Th(IV) radionuclides were quantitatively retained on the column at pH 6.0, eluted with 2.0 mL of 5% (v/v) HNO₃ and then determined by ICP-MS. The column possessed very selectivity to the radionuclides. No significant retention was noted from transition metals ions or rare earths metals by ICP-MS analysis. The developed method was validated by analysis of certified reference materials of Nearshore Seawater (CASS-4) and domestic sludge (SRM 2781), and successfully applied to the determination of U(VI) and Th(IV) in coastal seawater and estuarine water samples.
Isocyanates are a main component in the production of polyurethane (PUR) materials. Exposure to isocyanates can put workers at risk for respiratory disorders like “occupational asthma”. Dry air sampling devices were used in this field study of exposure assessment during the simulated spray paint coating. The dry air sampler allows sensitive and reliable detection coupled with convenient and safe use. The unique design of the dry sampler allows replenishment of the derivatization agent to the particle filter that allows longer sampling time (more than 8 hours). Low detection limits can be obtained by the analysis of urea derivatives by LC-MS-MS in the testing laboratory.

The sampling device will derivatize both the isocyanate monomers and oligomers. Monomers and polymers of isophorone diisocyanate (IPDI), and 1,6-hexamethylene diisocyanate (HDI) were detected in this study. HDI polymeric species included HDI-Isocyanurate, HDI-Uretidone and HDI-Biuret. The IPDI polymeric form IPDI-Isocyanurate was also detected. Quantitative analysis of the isocyanates was possible by using the available deuterated internal standards. The quantitative results indicated that HDI-Isocyanurate and IPDI-isocyanurate were present at the highest concentration in the air during spraying.

Keywords: Air, Environmental, Industrial Hygiene, Paint/Coatings
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Mercury is recognized as one of the most toxic elements in nature and its toxicity, bioavailability and transport is dependent on the specie. Organic mercury compounds are known as the most toxic species of mercury and show the highest bioaccumulation. The concentrations of the species in environmental samples can vary from really low concentrations in water (pg L\(^{-1}\)) to high concentrations in predator fish and cetaceans (mg kg\(^{-1}\)) and can be challenging for analytical scientists. Speciation in low concentrated samples is nearly impossible with most instrumentation without any preconcentration of the mercury species. In this work, we use an online preconcentration based on the strong interaction of mercury with sulfur compounds and the subsequent separation and analysis via HPLC-CV-AFS. The method was applied for water samples (e.g. urine, crude sewage, sea water) and MeHg\(^+\) was found in spiked samples with recoveries from 91 to 102 %. Different environmental relevant samples were digested/extracted to different protocols and analyzed with this method: Estuarine sediment ERM® CC-580, various marine materials (e.g. TORT-2, DOLT-4, DORM-2) and hair reference materials. Satisfactory recovery was found ranging from (90 - 105 %). Additionally, a method comparison was performed with the standard speciation method isotope dilution ID-GC-ICP-MS for rice samples and different tissues of long-finned pilot whales and good correlation coefficients (0.9663 R\(^2\) and 0.9728 R\(^2\), respectively) obtained. The developed method requires a minimal amount of sample pretreatment, is low in costs, achieves a LOD of 0.04 ng L\(^{-1}\) for MeHg\(^+\) producing reliable and good results.
The EPA proposed new rule (40 CFR, part 423) is an attempt to reduce the amount of toxic metals and other pollutants discharged to surface water from power plants. Previous regulations do not consider the additional burden of water discharge pollutants from air pollution control systems such as flue gas de-sulphurization (FGD), selective catalytic reduction (SCR) and flue gas mercury control (FGMC).

The main pollutants of concern include metals (e.g mercury, arsenic and selenium), nitrogen and total dissolved solids (TDS). Toxic metal discharges have a huge impact on the environment as they bio-accumulate in wildlife and cause a wide array of human health issues. The new ruling would establish new and additional requirements for wastewaters associated with FGD wastewater, fly and bottom ash transportation water, flue gas mercury control water, combustion residual leachates from landfill and surface impoundments, metal cleaning wastes and gasification wastewater. Depending on the final options the best available technology (BAT) that is economically achievable will need to be applied to reduce pollutant discharges. This is particularly challenging for dissolved forms of As, Se and Hg as they are not removed by surface impoundments.

PSA have a number of online and laboratory measurement based on AFS and sampling solutions to confirm the efficiency of the wastewater treatment processes and for compliance monitoring. These are outlined with recent experiences and data from the field.

Keywords: Environmental/Waste/Sludge, Mercury, Metals, Monitoring
Application Code: Environmental
Methodology Code: Process Analytical Techniques
Environmental Samples - Separation Techniques

**Abstract Title**
Improved Quantification Method of Organic Toxins for Human Health Assessment Using Direct Isotope Dilution Mass Spectrometry Gas Chromatography

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**Abstract Text**

In the past decade, an emerging branch of science called exposomics has shown that for man non-communicable diseases, the impact of the environment on the human body can account for as much as 90% of factors influencing onset and progression of diseases. This has given rise to toxin measurements in children and adults with diseases such as autism, ADHD, asthma, and many others. The ultimate goal of the new field of exposomics is to assess the effects of exposure on the epigenome and assess epigenetic modifications. Assessment of toxins and their origins has become a very important aspect of exposomics so that origin and dose, either acute and or chronic, from the air in the environment and indoor exposures can be measured and health implications assessed. To help answer exposomic questions, improved methods of solid phase adsorption have been developed in this work to quantify the volatile and semi-volatile toxins in outdoor and indoor air. Under the new methods, analyses of these solid phase extraction collections are performed with thermal desorption and direct isotope dilution (D-ID) mass spectrometry that uses no serial dilutions or external calibration curves. D-ID metrology facilitates calculation of analyte concentrations using a mathematical framework that utilize isotopic abundance ratios between the analyte and isotopically enriched standards. Examples of calibration curve versus direct isotope dilution thermal desorption GC-MS in an automated system is discussed and compared with traditional GC-MS analysis.

**Keywords:** Air, Elemental Mass Spec, GC-MS, Thermal Desorption

**Application Code:** Environmental

**Methodology Code:** Mass Spectrometry
Environmental Samples - Separation Techniques

Removal of Cr(VI) from Aqueous Solutions Using the Polyurethane Foam Like Adsorbent

Polyurethane form-like adsorbent (PUF) was successfully prepared using polypropylene glycol (PPG), 4,4'-Diphenylemethane diisocyanate (MDI) and Tri-n-octylamine (TnOA), and then PUF was impregnated with hydrochloric acid. The resulted adsorbent (HCl-PUF) used to remove Cr(VI) from environmental water samples, and Cr(III) was not adsorbed on HCl-PUF. Chromium exists widely in nature as both trivalent and hexavalent forms. Although, Cr(III) is an essential element for humans at trace levels, Cr(VI) has great toxicity and carcinogenicity at low concentrations. Cr(VI) is typically present in aqueous solutions, such as CrO$_4^{2-}$ anion. Therefore, HCl-PUF is an anion exchange adsorbent.

In this study, the preparation conditions of HCl-PUF and the adsorption conditions of Cr(VI) on HCl-PUF were investigated, such as the amounts of reagents, the adsorption time, the solution pH, and coexisting ions. Cr(VI) was quantitatively adsorbed on HCl-PUF at a pH range 1.0-6.0 from aqueous solutions and their adsorption isotherms were fitted by the Langmuir equation. The maximal adsorption capacity of Cr(VI) is $1.48 \times 10^{-3}$mg/g. The property of HCl-PUF was extended to the recovery of Cr(VI) in the environmental water samples.

Keywords: Adsorption, Environmental/Water, ICP, Solid Phase Extraction
Application Code: Environmental
Methodology Code: Separation Sciences

Session Title
Environmental Samples - Separation Techniques

Abstract Title
Removal of Cr(VI) from Aqueous Solutions Using the Polyurethane Foam Like Adsorbent

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Date: Tuesday, March 10, 2015 - Afternoon
Time: Room: Exposition Floor, Hall F, Aisles 390
Rice (Oryza sativa) is a staple food for many people and is grown around the world. In US it is grown in Gulf Coast States (Louisiana, Mississippi and Texas) as well as Arkansas, California, and Missouri. Many metals in low concentrations can present adverse health effects, and, hence, the concentration of metals in rice is important for risk assessment. This work presents the results and comparison of a study of selected metals (As, Cd, Cu, Cr, Fe, K, Mg, Mn, and Zn) in rice from Gulf coast states as well as a comparison from selected areas from around the world (India and Bangladesh). Results were determined using inductively coupled plasma-optical emission spectrometry as well as anodic stripping voltammetry for selected species of metals, particularly arsenic and chromium.

Keywords: Analysis, Atomic Emission Spectroscopy, Elemental Analysis, Environmental Analysis

Application Code: Environmental

Methodology Code: Atomic Spectroscopy/Elemental Analysis
In this presentation, the use of abandoned mine drainage (AMD) for irrigation of lettuce, sunflower, and tomato will be presented and discussed. The use of AMD for irrigation of crops has been explored; yet, much of the available information appears to deal with acid mine drainage and its treatment with lime to raise its pH to near neutral levels. The purposes of this investigation are: 1) to examine how the use of untreated, near-neutral-pH AMD from southwestern Pennsylvania for irrigation of tomatoes, lettuce, and sunflowers affects the growth of these plants; and 2) to determine the concentrations of iron, aluminum, manganese, and arsenic in the irrigated plants. A group of tomatoes, lettuce, and sunflowers are presently being grown at the Lowber, PA AMD passive treatment facility and irrigated with AMD from the settling ponds and wetlands. A second group of the same plants are currently being grown at the residence of one of the authors and watered with tap water spiked with 10 ppm each of Fe, Al, Mn, and As. Control plants, watered with uncontaminated water, are being grown in each group. The plants will be harvested, sectioned, wet-ashed, and analyzed for the aforementioned metals by flame (FAAS) and hydride generation (HGAAS) atomic absorption spectrometry. Results for Fe, Al, Mn, and As in the plants from each group grown in this study will be presented and discussed, as will plant growing and harvesting, sample preparation, analytical methods, and future plans for this project.

Keywords: Atomic Absorption, Environmental/Biological Samples, Environmental/Water, Metals
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Milk is heat-treated or pasteurized prior to distribution to destroy potentially dangerous bacteria. Heat treating milk also converts or degrades lactose to epilactose and lactulose. There are benefits to the presence of these breakdown products. First, they allow the milk industry to monitor carefully milk degradation. Second, the indigestible disaccharides have pharmaceutical benefits. For example, lactulose is used to treat constipation. Further, epilactose has beneficial prebiotic effects for the treatment mal-digestion. Thus, detailed chromatographic separation and analysis of these sugars are abundant in literature including the USP.

The HPLC separation of lactose, lactulose and epilactose is outlined in the United States Pharmacopeia Convention (USP). However, it is difficult to separate these saccharides completely.

Recently, the effective, simultaneous separation of lactose, lactulose, and epilactose was demonstrated using the new Shodex HILIC column (4.6 mm x 250 mm). The polymer based polyvinyl alcohol packing material is modified with tertiary amino functional groups to provide separation. The separation of these disaccharides takes a total of 15 minutes with resolution greater than 1.6. The calibration curves have demonstrated linearity between 500 µg/L and 10 g/L with RI detection.
Liquid Chromatography - Food Science, Bioanalytical and Biomedical

Portable Medium Pressure Capillary Liquid Chromatography based on a Modular Microfluidic System Using Off-the-Shelf Components

In contrast to trends towards miniaturised and portable instrumentation to be used out of the laboratory (on-site, in-field), portable liquid chromatography (LC) instrumentation for rapid on-site analyses has not progressed towards routine applications. In previous studies on portable LC, most of the designs relied strongly on in-house fabricated components, thus significantly limiting the availability of such portable systems for other users.

Here we investigated a design philosophy of a portable medium-pressure LC system based on using off-the-shelf easily accessible components. The basis of the system is a modular microfluidic platform with the components assembled in a flexible manner on a breadboard (Fig.1). The components are syringe pumps and switch valves, joined with capillaries and microfluidic fittings, complemented with other components, including an injection valve and on-capillary detectors, all controlled by a PC. The pumping system is formed by miniaturised syringe pumps (5, 20 and 100µL options). Two pairs of pumps were used for each mobile phase to enable gradient elution capability, connected with two microfluidic switching valves, and then connected together through a Y-connector, thus providing a low hold-up volume for gradient formation. A 4 or 20 nL nano-LC sampling valve was used for the injection system, connecting to a capillary LC column (100 µm i.d., 50-250 mm length, C18 RP monolith). Photometric detection was conducted through the capillary (100 µm i.d. PTFE-coated fused silica) with an LED-based UV-vis-NIR photometric detector, and/or an end-column electrochemical detector (amperometric and/or potentiometric). The characterisation of this portable LC system was conducted theoretically and experimentally, including the operating pressure, the performance in gradient mode and the performance of the detectors. The initial test analytes included primarily charged and uncharged food dyes of varying hydrophobicity.

Capillary LC, Detector, Lab-on-a-Chip/Microfluidics, Portable Instruments
Bioanalytical
Liquid Chromatography
Diabetes is a metabolomic disease that affects many people in the world and in the United States. One of the major health effects associated with diabetes is the elevated levels of glucose in the bloodstream, which can lead to non-enzymatic glycation of serum proteins such as human serum albumin (HSA). Sulfonylurea drugs are a class of anti-diabetic drugs used to treat diabetes. Second generation sulfonylurea drugs are most commonly used to treat type II diabetes because of their effectiveness and ease in excretion from the body. The purpose of this study was to use high-performance affinity chromatography (HPAC) to examine the binding of various second generation sulfonylurea drugs to HSA with various stages of in vitro glycation. Frontal analysis was used to provide data on the overall affinity and number of binding sites for HSA and glycated HSA with various sulfonylurea drugs. Zonal elution competition studies were used to detect any changes in site-specific interactions that may have occurred as a result of glycation for the same sulfonylurea drugs at major drug-binding sites on HSA. The results of these studies should aid researchers in pharmaceutical development and in personalized medicine for the treatment of diabetes, as well as provide a better understanding of how the metabolomic effects of diabetes can affect drug binding in the body.

Keywords: Bioanalytical, HPLC, Metabolomics, Metabonomics
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
### Abstract Text

In this study, the effect of random versus site-directed immobilization techniques on the performance of antibody-based HPLC columns was investigated using a single-domain camelid antibody (VHH) directed against methotrexate as a model system. First, the high flow-through support material POROS-OH was activated with disuccinimidyl carbonate (DSC), and the VHH was bound via random amines located on the protein's surface. The resulting column was characterized by Frontal Affinity Chromatography (FAC). Then, two site-directed techniques were explored that were expected to increase column efficiency by immobilizing the antibody via its C-terminus, i.e., away from the antigen-binding site. In one approach, a poly-lysine tail was added, and the antibody was immobilized onto DSC-activated POROS. In the second site-directed approach, the VHH was modified with the Avitag peptide, and a biotin-residue was enzymatically incorporated at the C-terminus. The biotinylated antibody was then immobilized onto Neutravidin-derivatized POROS. While FAC analyses showed that both of the site-directed approaches yield better results than the random immobilization, the by far highest efficiency was determined for the immunoaffinity column based on Avitag-biotinylated antibody.

### Keywords
- HPLC Columns
- Immobilization
- Optimization
- Protein

### Application Code
- Biomedical

### Methodology Code
- Liquid Chromatography
Contamination and adulteration of heparin (HEP) have elevated the importance of separation and detection of glycosaminoglycans (GAGs) to ensure product quality and safety. Being a natural product, HEP extracts can contain other GAGs, such as chondroitin sulfate (CS) or dermatan sulfate (DS) but unnatural adulterants such as oversulfated chondroitin sulfate (OSCS) must also be checked. The current HPLC/UV method, established by the FDA and USP, for separating and detecting HEP and other GAGs is performed on a low capacity strong anion exchange column with gradient elution using a concentrated sodium perchlorate eluent. Despite the ability to separate HEP from OSCS, this method tends to suffer from long run times and broad peaks. Previously, we have shown ion exclusion chromatography can be effective for the separation of hyaluronic acid (HA) from either HEP, CS, or DS. Resolution of HEP and OSCS was incomplete. To the best of our knowledge, weak anion exchange (WAX) chromatography has not been explored in depth to separate HEP from OSCS and other GAGs. We adopted for use a commercially available 4.6 mm x 50 mm column packed with 5 micron DEAE derivatized methacrylate mono-disperse porous polymer particles. The following gradient mobile phase of initially 80% A [2.6 mM sodium di-hydrogen phosphate (pH 3.0)]-20%B [1 M sodium perchlorate in A] was changed to 10% A-90% B in 60 min at various flow rates ranging from 0.2 to 0.8 mL/min with detection at 202 nm. Using 0.8 mL/min, resolution of HA, CS or DS, HEP, and OSCS was possible in less than 30 min. HA, evident as a sharp peak, eluted in less than 1 min, CS or DS and HEP were resolved at about 12 and 17 min, respectively, and OSCS was retained at 29 min but with a baseline peak width of less than 6 min. Lowering the flowrate did not change peak resolution significantly. Raising the pH to change the ion exchange capacity of the column and changing the type of weak anion exchange column are under study.

Keywords: Chromatography, Ion Exchange, Pharmaceutical
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Little cigars and cigarillos (LCC) are widely available in the U.S. and historically have been less costly than cigarettes. Unlike cigarettes, LCC are offered in a variety of flavors including vanilla, wine, cherry, and raspberry, which could appeal to novice smokers. During manufacturing, a variety of chemical additives are added to the tobacco to affect the flavor, taste, and marketability of the products. Ammonia is an additive that may increase nicotine bioavailability and addiction potential by boosting the relative amounts of the free-base form of nicotine in smoke, as well as increasing the “harshness” or “mouth feel” typical of cigar smoke. For this study, tobacco from 24 brands of LCCs was analyzed for ammonia content. Ammonia levels were determined using ion chromatography coupled with conductivity detection. Of the 24 brands, ammonia levels in little cigars and cigarillos spanned 0.8 to 3.7 mg/g. This range of ammonia is similar to reported values for cigarettes (0.15 to 2.4 mg/g). Despite their visual-physical differences, LCCs and traditional cigarettes are similar with respect to ammonia levels in the brands tested. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.
Eggshell membrane is a novel dietary supplement containing naturally occurring glycosaminoglycans and fibrous proteins such as collagen type I that has a potential of becoming a new natural therapeutic for JCT (joint or connective tissue) disorder. Since collagen type I is a primary constituent of the eggshell membrane, quantitation of collagen in raw materials and finished dietary supplements could serve as an appropriate quality control measure. The analytical methodology for determination of collagen in eggshell membrane is very limited; therefore we developed and validated a simple method for analysis of collagen in raw materials and finished dietary supplements. Collagen containing products are hydrolyzed in sulfuric acid using microwave digestion. The hydrolyzed material is then diluted in water and derivatized with 6-Aminoquinoline-N-hydroxy-succinimidyl carbamate. Amino acids are separated using reverse phase chromatography with UV detection at 260 nm. Target amino acid hydroxyproline is quantitated using a primary grade standard. Based on the amount of hydroxyproline, collagen content can be determined using known/published conversion factors.

Keywords: Amino Acids, Bioanalytical, Derivatization, HPLC
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Low-molecular-weight biothiols play important roles in biological processes. Although many analytical methods for biothiols were developed, simultaneous determination of multiple biothiols has not been successful because of the difficulty in retaining biothiols on reversed phase chromatography (RLC). Hence, in this study, we developed an analytical method for multiple biothiols based on HPLC-fluorescence detection under hydrophilic interaction chromatography (HILIC) conditions, where the retention and separation of highly polar compounds is easily achieved. Thiols were derivatized with thiol-selective fluorogenic reagent, ammonium 7-fluoro-2,1,3-benzoxadiazole-4-sulfonate (SBD-F). Among the six HILIC columns examined (bare silica, amide, amino, diol, phosphorylcholine, and sulfoalkylbetaine columns), the sulfoalkylbetaine column proved to be the best for the SBD-thiols separation. Eight thiols ([i]N-acetylcysteine, cysteamine, homocysteine, cysteine, cysteinylglycine, glutathione, [gamma]-glutamylcysteine, and internal standard, [i]N-(2-mercaptopropionyl)glycine) were well separated within 10 min. The detection sensitivity was 30-300 times higher compared to RPC methods, partly due to the enhancement in the SBD-thiol fluorescence owing to the acetonitrile-rich mobile phase used. The method successfully quantified six biothiols in a human plasma sample with good precisions. This method should be a useful tool for investigating the relationship between sulfur metabolism and related diseases.
Coumarin is an active principle found in several vegetable species with a characteristic smell of vanilla. It is widely used as a perfume fixer, paint and spray additive, skin-care-related consumer products and in cleaning products. It also possesses clinical value due to antibiotic and analgesic properties, besides its potential use in the treatment of cancer and AIDS(1,2). High temperature liquid chromatography (HTLC) deal to liquid chromatography separations performed at elevated temperatures using organic solvent-water mixtures as the mobile phase. The consumption of organic solvent in HTLC mobile phase is considerably reduced with increasing temperature (3). In this study, 1-naphthylamine (NA) attached PHEMAH (NA-PHEMAH) beads were prepared by diazotization of 1-naphthylamine (NA) and covalent coupling of it to the PHEMAH beads for stationary phase. Then Vaseline cocoa butter lotion sample was separated on PHEMA column by using HTLC. HTLC results revealed that NA-PHEMAH stationary phase yielded reasonable separations of skincare cream with symmetrical peak.

References

Acknowledgements
This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK): grant number KBAG-112T336.

Keywords: Bioanalytical, Chromatography, High Temperature, HPLC
Application Code: Bioanalytical
Methodology Code: Other
Non-mammals, such as *Tribolium castaneum* - flour beetle, *Danio rerio* – zebrafish, and *Aiptasia pallida* – sea anemones, offer new model systems and thus new challenges for the measurement of signaling molecules. To further understand the molecular changes of amino acid and phytoestrogen release associated with behavior, separation methodologies were developed for high-performance liquid chromatography. Amino acid neurotransmitters were derivatized by bonding an alkylthiol-isoindole to the amino acid amine using O-phthaldialdehyde (OPA) and sodium sulfite. The high pH sensitivity of each derivative limited the use of phosphate buffers, typically used at neutral pH. Instead, the effects of a citrate-acetate based mobile phase were investigated to not only allow resolution of these species but also increase their sensitivities when using an electrochemical-based detection system. For the detection of phytoestrogens such as isoflavones, which have been known to affect reproduction of sea life, solid phase extraction and a formic acid-acetonitrile gradient for high performance liquid chromatography was developed. Three isoflavones: diadzein, genistein, and biochanin A were measured in sea waters containing symbiotic and aposymbiotic sea anemones. It is hypothesized that the symbiotic relationship between these anemones and their algal counterparts may regulate the method sea anemones reproduce, specifically the algae release compounds that decide whether the anemones reproduce sexually or asexually. It is hoped that the methods described here will provide greater insight into how behavior is affect by the development of signaling pathways in these species.

**Abstract Text**

Non-mammals, such as *Tribolium castaneum* - flour beetle, *Danio rerio* – zebrafish, and *Aiptasia pallida* – sea anemones, offer new model systems and thus new challenges for the measurement of signaling molecules. To further understand the molecular changes of amino acid and phytoestrogen release associated with behavior, separation methodologies were developed for high-performance liquid chromatography. Amino acid neurotransmitters were derivatized by bonding an alkylthiol-isoindole to the amino acid amine using O-phthaldialdehyde (OPA) and sodium sulfite. The high pH sensitivity of each derivative limited the use of phosphate buffers, typically used at neutral pH. Instead, the effects of a citrate-acetate based mobile phase were investigated to not only allow resolution of these species but also increase their sensitivities when using an electrochemical-based detection system. For the detection of phytoestrogens such as isoflavones, which have been known to affect reproduction of sea life, solid phase extraction and a formic acid-acetonitrile gradient for high performance liquid chromatography was developed. Three isoflavones: diadzein, genistein, and biochanin A were measured in sea waters containing symbiotic and aposymbiotic sea anemones. It is hypothesized that the symbiotic relationship between these anemones and their algal counterparts may regulate the method sea anemones reproduce, specifically the algae release compounds that decide whether the anemones reproduce sexually or asexually. It is hoped that the methods described here will provide greater insight into how behavior is affect by the development of signaling pathways in these species.

**Keywords:** Amino Acids, Bioanalytical, HPLC, Neurochemistry

**Application Code:** Bioanalytical

**Methodology Code:** Liquid Chromatography
A study on the retention behavior of pyrazine derivatives in reversed-phase liquid chromatography (RPLC) was conducted. The retention factors of pyrazines were measured with changing column temperature. An abnormal temperature effect of the pyrazines' retention was observed, where the retention factors of all alkylpyrazines studied were increased with increasing column temperature on an octadecylsilica (C18) stationary phase. Non-linear van't Hoff plots were obtained for some pyrazines, indicating that the retention mechanisms for the pyrazines were not constant over the temperature range studied (20-60[degree]C). Similar trends were also observed on all other stationary phases including butylsilica (C4), octylsilica (C8) and triacontylsilica (C30) phases. In contrast, no similar temperature-dependence was found with methanol/water mobile phases, suggesting that the abnormal temperature effect on the retention of the pyrazine derivatives might be mainly induced by acetonitrile-based mobile phases. The results contribute to a better understanding of the retention mechanism of pyrazines in RPLC.

**Keywords:** HPLC, Temperature

**Application Code:** Food Identification

**Methodology Code:** Liquid Chromatography
Vitamins play vital roles in the metabolic activities of the body. However, because they cannot be synthesized in sufficient quantities in the body, if at all, they must be ingested either in our meals or by other means, such as tablets. Vitamins can be classified into two groups, water-soluble and fat-soluble, and their analysis is generally conducted by high-performance liquid chromatography. The water-soluble type consists mainly of highly polar basic components which exhibit weak retention in analysis by reversed-phase liquid chromatography. Since they exhibit weak retention, the analysis is typically conducted using ion-pair reagents. That makes it difficult to apply gradient elution. On the other hand, the fat-soluble type consists of hydrophobic components. They are usually analyzed by reversed-phase liquid chromatography with organic solvent mobile phases or normal-phase liquid chromatography.

In this study, a Shim-pack MAqC-ODS I was used to analyze water- and fat-soluble vitamins in a single method. This column contains ODS-modified and metal-doped stationary phase, so it was able to retain the weakly-retained components without ion-pairing reagents. In addition, by using a 100% organic solvent mobile phase after water-soluble vitamins were eluted, fat-soluble vitamins were also able to be analyzed.
We developed an easy-to-use analytical method for amyloid fibrils by high-performance liquid chromatography (HPLC) with post column fluorescent labeling. Thioflavin T (Th T), which was used as a labeling agent for amyloid fibrils, is a traditional probe for the confirmation of amyloid fibril formation. Amyloid fibrils plays important roles in several neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. Although it had been previously known that the amyloid fibril formation takes place as a result of conformational change of the proteins from a stable α-helical structure into a β-sheet structure, the details of the amyloid fibril formation process are not completely understood. The traditional fluorescence spectroscopy measurements is used for monitoring the aggregation reaction, but only the total amount of the amyloid aggregates such as amyloid fibrils and fibrillar oligomers were measured in this method. In order to understand the fibril formation process, the development of an efficient method enable to separate and detect the amyloid aggregates is required.

In this work, the Th T labeling of the amyloid fibrils in sample solution was performed following the separation of the different-sized amyloid fibrils by a reversed-phase column with the mobile phases containing hexafluoroisopropanol. The fluorescence was monitored at 482 nm, with a maximum excitation wavelength at 450 nm. The developed method detected overlapping peaks of the amyloid fibrils within 3 min. In addition, the novel method showed its sensitivity for the detection of the amyloid aggregates was several-fold higher as compared to the traditional fluorescence spectroscopy measurements. These results suggest that this analytical method may be used to analyze the amyloid fibrils and identify an inhibitor of the fibril formation.

Keywords: Bioanalytical, HPLC Detection, Spectrometer
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
A new method has been developed for determining the organic impurities in Ext. D&C Violet No. 2 (monosodium salt of 1-hydroxy-4-(o-sulfo-p-toluidino)anthraquinone). The color additive is listed under 21 CFR 74.2602a for use in externally applied cosmetics and is required to be batch certified by the U.S. Food and Drug Administration (FDA). The impurities determined are the intermediate starting materials (p-toluidine, 1-hydroxyanthraquinone, and 1,4-dihydroxyanthraquinone); subsidiary colors (mainly the disodium salt of 1,4-bis(o-sulfo-p-toluidino)anthraquinone); and sodium salts of sulfonated p-toluidines. The new method uses ultra-performance liquid chromatography (UPLC) with a 1.7-μ particle size C-18 column, 0.1 M ammonium acetate and acetonitrile as eluants, and photodiode array detection. The method was validated by measuring linearity, accuracy, precision, and limit of detection for each impurity. Six-point linear regression calibration curves had r² values that were all greater than 0.999. Recoveries of impurities were 99 to 101%. Samples from 20 previously certified lots of Ext. D&C Violet No. 2 were analyzed with the new method, and the results are comparable with the results previously obtained by gravity column chromatography, thin-layer chromatography, and isooctane extraction. The new UPLC method determines all of the impurities in a single analysis, uses green technology by reducing organic waste generation, and saves time and labor.

Keywords: HPLC, Quantitative, Validation

Application Code: Food Safety

Methodology Code: Liquid Chromatography
Carbohydrates are important food components affecting taste and nutrition. The determination of the types and levels of carbohydrates in foods is important for energy evaluation, nutritional labeling, quality control and for identifying any possible product adulteration.

Carbohydrate analysis can be problematic since they are very polar compounds, exhibit similar structural characteristics and do not have a suitable chromophore. Their analysis can be performed after suitable derivatization using techniques such as gas chromatography, capillary electrophoresis, HPLC with ultraviolet (UV) or Fluorescence (FL) detection or HPLC with mass spectrometry (MS). These methods utilize derivatization to improve the chromatographic resolution and detector sensitivity; however they can lead to increased assay variability. Direct methods that do not require derivatization include HPLC with refractive index (RI), High-Performance Anion-Exchange Chromatography with Pulsed Amperometric detection (HPAE - PAD) using a high pH mobile phase or HILIC mode chromatography with charged aerosol detection. This poster presents approaches for carbohydrate analysis that solve the challenges for separation and detection of carbohydrate analysis without requirement for laborious derivatization: HPLC with pulsed amperometric detection (HPLC-PAD), HPLC with charged aerosol detection (HPLC-CAD).

When the PAD option on the electrochemical detector is coupled with a gold working electrode, it provides high sensitivity and selectivity for the measurement of carbohydrates in complex food sample matrices. The charged aerosol detector is a mass-sensitive detector that can measure all non-volatile, and many semi-volatile compounds in a sample. The method has a limit of detection of <10ng, on column and a wide dynamic range that covers nanogram to microgram levels with high reproducibility. Application examples for various juice samples, milk and other dairy products, syrup and honey will be discussed.

Keywords: Beverage, Carbohydrates, Food Science, Liquid Chromatography
Application Code: Food Science
Methodology Code: Liquid Chromatography
There is considerable interest in botanical supplements due to their purported health benefits. Mangosteen (Garcinia mangostana L) is a tropical fruit that is indigenous to Southeast Asia, where it has been historically used to treat abdominal pain, diarrhea, dysentery, inflammation, wound infection, suppuration, chronic ulcer and as a homeopathic therapy in the treatment of Parkinson's disease. Such therapeutic benefits have been mostly attributed to a unique family of compounds referred to as xanthones that are most abundant in the pericarp of the fruit. Five major xanthones present in mangosteen pericarp, include \textit{\textsuperscript{\textregistered}}-mangostin, 3-isomangostin, gartanin, 8-desoxygartanin and 9-hydroxycalabaxanthone.

Chromatographic analysis of the xanthones and other important analytes contained in this supplement presents is challenging. Reversed phase HPLC with UV detection is widely used for the analysis of xanthones, but such methods lack analyte resolution and/or require exceedingly long analysis time. Improving these issues, by using UHPLC may still require >25 mins to complete since the flow rates required to achieve optimal column efficiency generate exceedingly high back pressures, typically beyond UHPLC system limits. This situation is addressed by the new Vanquish UHPLC system, which consists of a binary parallel pump capable of operating at pressures up to 1500 bar, autosampler and diode-array detection.

Consequently, UHPLC columns containing smaller particle size can be operated at high flow rates to improve analyte resolution and sample throughput. The analytical power of the Vanquish system is typified by the improvements in analyte resolution and reduced run time shown for the analysis of mangosteen pericarp.

Keywords: Biomedical, Liquid Chromatography, Natural Products

Application Code: Biomedical

Methodology Code: Liquid Chromatography
**Abstract Text**

Flavan-3-ols, known also as proanthocyanidins or condensed tannins, after lignans are the second most common class of natural phenolic substances found in nature. Single linked, B-type procyanidins occur in many foods, with those from apples and cocoa being the most prominent in the western diet. A possible connection between procyanidins and cardiovascular disease is particularly interesting. The double linked, A-type procyanidins occur in cinnamon and cranberries and are believed to contribute to anti-diabetic and anti-UTI properties of these foods. Procyanidins form chains containing polymerized subunits of epicatechin and catechin, with the number of subunits being between 2 and 30. With the ever increasing interest in determining their significance as dietary antioxidants and pharmacologically active components, better analytical techniques allowing their standardization in dietary supplements are required. We developed a sensitive and selective gradient HPLC method combining photodiode array and coulometric electrochemical array detection to resolve and quantify numerous procyanidins in different foods/supplements. This approach also enabled the estimation of standard purity and abundance of contaminants. A second method, using charged aerosol detection, was used to determine the presence of impurities that either lack a chromophore or are not electrochemically active. A third method was developed using gradient UHPLC with UV detection. This approach significantly shortened the analysis time from 30 to <5 minutes, while maintaining analyte resolution.

**Keywords:** Food Contaminants, Food Science, HPLC Detection, Natural Products

**Application Code:** Food Science

**Methodology Code:** Liquid Chromatography
It has been estimated that less than 1% of all bacteria have been cultured in the laboratory environment. To understand the intricacies of microbial ecology, we need isolates that can be easily characterized and cataloged. Traditional methods for characterization utilize biochemical methods and molecular techniques allowing for identification down to the species level. However, most of these methods are either too costly or not rapid enough for analysis of large groups of isolates. This work sought to develop a reverse phase HPLC method for identification of pink bacterial carotenoids unique to the genera [i]Deinococcus[/i] and [i]Hymenobacter[/i]. [i]Deinococcus[/i] strains are the only known producers of deinoxanthin lending to the notion that the presence of deinoxanthin suggests that the strain must be [i]Deinococcus[/i]. [i]Hymenobacter[/i] strains are known to produce many derivatives of the carotenoid flexixanthin, with 2'-hydroxyflexixanthin being the common carotenoid observed. Bacterial cultures were isolated from submerged freshwater substrates. Pink colonies were then isolated for testing and analysis. To verify the identity of putative [i]Deinococcus[/i] and [i]Hymenobacter[/i] species, 16S-rRNA analysis was used. A total of 122 isolates were analyzed using our method resulting in positive identification of 7 deinoxanthin and 72 2'-hydroxyflexixanthin producing bacteria with 43 isolates exhibiting unidentified carotenoids. The method developed was highly effective in not only determining the genus of a given isolate, but also in denoting chromatogram differences indicative of species divergence, such as additional minor carotenoids. Future work will focus upon expanding our method to search for carotenoids unique to other genera.

Abstract Text

Abstract # 1550-9

Session Title: Liquid Chromatography - Food Science, Bioanalytical and Biomedical

Abstract Title: Novel Method for Identification of [i]Deinococcus[/i] and [i]Hymenobacter[/i] Using High Performance Liquid Chromatography

Primary Author: Thomas Kuborn

University of Wisconsin Oshkosh

Co-Authors: Kevin Crawford, Patrick Klepp, Sabrina Mueller-Spitz

Abstract Text

It has been estimated that less than 1% of all bacteria have been cultured in the laboratory environment. To understand the intricacies of microbial ecology, we need isolates that can be easily characterized and cataloged. Traditional methods for characterization utilize biochemical methods and molecular techniques allowing for identification down to the species level. However, most of these methods are either too costly or not rapid enough for analysis of large groups of isolates. This work sought to develop a reverse phase HPLC method for identification of pink bacterial carotenoids unique to the genera [i]Deinococcus[/i] and [i]Hymenobacter[/i]. [i]Deinococcus[/i] strains are the only known producers of deinoxanthin lending to the notion that the presence of deinoxanthin suggests that the strain must be [i]Deinococcus[/i]. [i]Hymenobacter[/i] strains are known to produce many derivatives of the carotenoid flexixanthin, with 2'-hydroxyflexixanthin being the common carotenoid observed. Bacterial cultures were isolated from submerged freshwater substrates. Pink colonies were then isolated for testing and analysis. To verify the identity of putative [i]Deinococcus[/i] and [i]Hymenobacter[/i] species, 16S-rRNA analysis was used. A total of 122 isolates were analyzed using our method resulting in positive identification of 7 deinoxanthin and 72 2'-hydroxyflexixanthin producing bacteria with 43 isolates exhibiting unidentified carotenoids. The method developed was highly effective in not only determining the genus of a given isolate, but also in denoting chromatogram differences indicative of species divergence, such as additional minor carotenoids. Future work will focus upon expanding our method to search for carotenoids unique to other genera.

Keywords: Bioanalytical, Chromatography, HPLC, Liquid Chromatography

Application Code: Bioanalytical

Methodology Code: Liquid Chromatography
<table>
<thead>
<tr>
<th>Session Title</th>
<th>Evaluation of a Novel 96-Well Filter Plate for the Effective Removal of Serum Protein and Phospholipids Prior to LC-MS/MS Analysis</th>
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<tr>
<td>Primary Author</td>
<td>Elena Gairloch</td>
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<tr>
<td>Co-Author(s)</td>
<td>Frank Kero, Lee Williams, Martin Cherrier, Victor Vandell</td>
</tr>
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**Abstract Text**

When analyzing serum or plasma, the very nature of the matrix can cause problems with the analytical methodology. Endogenous components such as salts, proteins and phospholipids are all present and can have a marked effect on instrument response in terms of ion suppression or enhancement effects. This variation in signal can lead to quantitation issues and method reliability problems. In recent years a number of filter plates have been developed to address these specific interferences. This poster evaluates the performance of a novel 96-well filter plate for the simultaneous removal of proteins and phospholipids prior to LC-MS/MS analysis. Protein removal was compared via gel electrophoresis using a NuPAGE Novex 12% Bis-Tris mini gel with MOPS SDS running buffer. Phospholipids and recovery and signal intensity for a variety of acidic, basic and neutral analytes were analyzed using a Waters 2795 liquid handling system coupled to a Quattro Ultima Pt triple quadrupole mass spectrometer. Analyte monitoring involved positive ion acquisition using electrospray ionisation in the MRM (multiple reaction monitoring) mode. For each analyte protonated molecular ions were fragmented to the most intense product ion. Phospholipid analysis involved monitoring MRMs of the most intense phospholipid ions (selected from full scan, SIR and precursor ion monitoring), fragmenting to the common 184 production.

Rough protein quantitation of the precipitated extracts showed greater than 99% protein removal for a 1:3 matrix:ACN crash ratio. Phospholipid free extracts were obtained when using 1:3 or 1:4 matrix:ACN crash ratios. Using 1% formic acid in ACN as the precipitant also resulted in good removal of phospholipids. Recovery experiments for a variety of analytes with varying logP and pKa values demonstrated recoveries greater than 60%. Full results, discussion and conclusions will be presented in the final poster.

**Keywords:** Liquid Chromatography, Mass Spectrometry, Sample Preparation, Solid Phase Extraction

**Application Code:** Bioanalytical

**Methodology Code:** Sampling and Sample Preparation
### Session Title
Sampling and Sample Preparation Techniques

### Abstract Title
Understanding the Health Safety and Environmental Implication of Formulating Solutions in the Laboratory Environment

<table>
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<tr>
<th>Primary Author</th>
<th>Anthony R. Kemperman</th>
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<td>Honeywell, Burdick and Jackson</td>
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#### Abstract Text
Laboratories are tasked with making blended formulations for use in environmental, pharmaceutical and biopharmaceutical applications for use with HPLC, gas chromatography and LC-MS. These formulations are often developed without a full Health Safety and Environmental (HS&E) review. The hazards can come from the neat chemicals used to prepare the formulation, blend or buffer. They can also come from the new hazards associated with the formulation. HS&E groups tend to focus on the neat chemical or ingredient used to make the formulation. HS&E issues from the process of making buffers and chemical solutions can reduce productivity and create hidden cost within a company.

The poster will examine the hazards associated with preparing formulations in the lab. One focus will be the hazards associated with the neat components used to make formulations. The other focus will be on the lesser understood hazards of the new formulation for use in an application. These unknown dangers can have a direct impact to worker safety, chemical inventory management and productivity. A solution to this dilemma is to utilize commercially prepared formulations. Honeywell LabReady Blends by Burdick & Jackson is an example of an offering that is specifically suited to address these factors. When organizations choose to “buy” versus “make” their formulations, they are able to have a clearly defined product that meets the various requirements of governing bodies of lab chemical hygiene. The poster will demonstrate how adopting Honeywell LabReady Blends can decrease the hazards of preparing formulations in-house. The poster will also show how risk can be mitigated through the purchasing of commercially prepared formulations.

#### Keywords
Chemical, Optimization, Solvent, Standards

#### Application Code
Safety

#### Methodology Code
Sampling and Sample Preparation
Glyphosate is part of a group of herbicides referred to as phospho-herbicides and is the most commonly used herbicide worldwide with around 25% of the global herbicide market. In 2001, the EPA estimated that Glyphosate was the most commonly used active ingredient in pesticides with between 85 to 90 million pounds applied per year. It undergoes rapid microbial degradation in plants, soil and water to the metabolite aminomethylphosphonic acid (AMPA). In Europe, the following limit values were inferred for glyphosate: ADI (acceptable daily intake): 0.3 mg/kg of bodyweight; AOEL (acceptable operator exposure level), systemic: 0.2 mg/kg bodyweight/day. In addition, Codex alimentarius has defined a MRL (maximum residue limit) of 0.05mg/Kg in meat or milk and 30mg/Kg in cereals.

As very polar molecules, the analysis of Glyphosate and AMPA is still a challenge. Indeed, they are difficult to extract with organic solvents and common solid phase extraction (SPE) sorbents. A new SPE sorbent based on Molecularly Imprinted Polymers (MIP) was developed for Glyphosate and AMPA. A MIP is a synthetic material with artificially generated three-dimensional network which shows affinity for a target molecule. SPE results have shown a good ability of the sorbent to catch both molecules with good recoveries in elution without salts interference problem in different water matrices (mineral water; natural groundwater) and FOOD and FEED cereals matrices. This sorbent will also be tested in the framework of the development of integrative passive samplers (POCIS) for Glyphosate & AMPA.
Improved Extraction Efficiency of Existing Pesticide Residues in Food via Automated Shaking During the QuEChERS Procedure

In the past decade, QuEChERS procedures have become standard methods for analysis of pesticides due to their cost-effectiveness and excellent performance. In many cases, the methods were developed and validated using spiked samples. However, evaluation of the methods for extraction of existing pesticide residues is necessary to fully confirm the methods’ performance. In this work, the QuEChERS procedure was performed for the extraction of existing pesticide residues from a number of produce and/or food samples. The shaking of the samples was done either by hand for 1 minute, as written in QuECHERS methods, or by using a simple shaker/vortexer for 10 minutes. The extraction efficiency for both procedures was compared. The use of the shaker resulted in 10-15% higher yield of existing pesticides for the tested food samples. Introduction of the simple and inexpensive shaker to the laboratory workflow can result in significant improvement in pesticide yields and measurement accuracy.

Abstract Text

Keywords: Food Contaminants, GC-MS, Pesticides, Sample Handling/Automation
Application Code: Food Contaminants
Methodology Code: Sampling and Sample Preparation
We invented a QuEChERS salts tablet, which made the QuEChERS extraction method for pesticides in fruits and vegetables even easier as regards dispensing the phase-separation salts. However, difficulties in making the tablet inhibited its production. Recent breakthroughs in the manufacturing process on a bench scale have enabled a better performing tablet that is easily handled and yields quantitative results comparable to commercially available QuEChERS salt packets. This presentation will share some promising results from this research.
Extraction techniques are plentiful; however, determining which technique to implement for analysis can be difficult. Percent recovery, selectivity, ease of extraction, and ruggedness, must all be considered. It is the goal of this study to investigate three different extraction methods: QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe), IL-SDME (ionic liquid single drop microextraction), and SPME (solid phase microextraction). In this discussion, the use of QuEChERS will be emphasized. QuEChERS is a liquid-liquid microextraction combined with a dispersive solid phase extraction cleanup. Primarily used for the extraction of pesticides from food products, QuEChERS has not yet been thoroughly investigated for forensic samples. This study will serve to determine if QuEChERS is a viable extraction method for the analysis of drugs in urine as well as compare this extraction method to the use of IL-SDME and SPME. In IL-SDME an ionic liquid drop is suspended above the sample until such a time that equilibrium has been reached. The drop is then desorbed in the GC inlet. This method is very similar to that of SPME except for the use of an ionic liquid drop rather than a coated fiber as the extraction media. The optimization of these techniques for the extraction of amphetamine, methamphetamine, morphine, benzoylecgonine, methadone, oxazepam, secobarbital, phencyclidine, 11-nor-9-Carboxy-9-THC, and nortriptyline from urine will be discussed as well as the sensitivity and selectivity of the method via gas chromatography-mass spectrometry (GC-MS).

Keywords: Forensics, Gas Chromatography/Mass Spectrometry, Sample Preparation, Toxicology
Application Code: Clinical/Toxicology
Methodology Code: Sampling and Sample Preparation
Chloride occurs naturally in crude oil, since petroleum is in constant contact with saline waters and sediments that contain large concentrations of this anion. Therefore, the concentration of chloride (reported as NaCl) in crude oils depends on the geographical region where it is deposited and also on the oilfield region where it is found. The presence of chloride in crude oil can lead to the formation of HCl during refining, which provokes corrosion of metallic pipelines and other units. Additionally, chloride can deactivate Ni- and Pd-based catalysts widely used in catalytic cracking. This work proposes a novel methodology for the determination of chloride in crude oil samples by ion chromatography (Dionex ICS-2100 equipped with an AS15 column) employing the extraction induced by emulsion breaking (EIEB). In fact, we are proposing a new way to perform EIEB, in which oil-in-water emulsions are employed instead of oil-in-water ones. The method was based on the formation of w/o emulsions between crude oil (1.0 g) with a Triton X-114 solution (5 mL). After formation, the emulsions were broken and the aqueous phases containing the extracted chloride were collected for analysis by IC. Several parameters that could affect the performance of the method were evaluated. Under optimum conditions, a 2.5% m/v Triton X-114 solution must be used for emulsification with 0.5 g of crude oil diluted with 0.5 g of mineral base oil. The emulsion must be agitated for 60 min before its breaking. After breaking by centrifugation, the extracts were filtered through a 0.2 [micro]m pore diameter membrane and diluted to fit the analytical curves (0.05 to 2.5 mg/L). The optimized method was applied in the determination of NaCl in five samples of Brazilian crude oils and the results were in good agreement with those obtained by the ASTM D6470 method. Recovery tests were also performed, yielding recovery rates in the range of 78 - 108%.

Keywords: Ion Chromatography, Petroleum, Sample Preparation
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Sampling and Sample Preparation
The demand for analysis of drugs of abuse in human urine, plasma and blood continues to grow each year throughout the world. As this demand increases many forensic and clinical laboratories struggle to analyze the large number of samples submitted and often seek high-throughput methodologies in order keep up with the workload. Quantitative determination for drugs of abuse in urine is commonly conducted by a solid phase extraction (SPE) to clean-up and concentrate the sample followed by LCMS analysis. The SPE sample preparation is normally the bottle neck in this process and often represents a significant portion of the time and man hours required for the analysis. The PerkinElmer[registered] Zephyr[registered] is a compact liquid handling workstation that can be specifically configured for SPE on the 96-well plate format for high-throughput parallel sample preparation. We have developed a series of SPE methods for drugs of abuse classes such as opiates, benzodiazepines, cannabinoids and amphetamines as well as the common metabolites in urine to demonstrate the time savings and precision of the Zephyr[registered]. These methods utilize the PerkinElmer[registered] Supra-Clean[circumflex O] spherical silica and Supra-Poly[circumflex O] spherical polymeric SPE sorbents packed in 96-well plates to produce excellent recoveries and reproducibility. Final analysis of the samples was conducted on the PerkinElmer[registered] SQ300[circumflex O] LCMS.
### Session Title
Sampling and Sample Preparation Techniques

**Abstract Title**
Automating Liquid-Liquid Extractions Using a Bench-Top Workstation

**Primary Author**
Edward Pfannkoch
GERSTEL, Inc.

**Co-Author(s)**
Fredrick Foster, Jacqueline Whitecavage, John Stuff

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**Abstract Text**

Liquid-liquid extractions have long been performed manually and are used to extract and concentrate analytes from aqueous matrices. Inclusion of liquid-liquid extraction in many official methods attests to the wide acceptance of the technique. Following solvent extraction it is also common to include an evaporation and reconstitution step to improve detection limits or exchange solvents for compatibility with subsequent chromatographic separations. Modern analytical labs are looking to automation to help reduce solvent usage and increase sample throughput while ensuring the high quality of the resulting data.

A single robotic X-Y-Z coordinate autosampler commonly used for sample introduction in GC or HPLC can be used to perform a wide variety of sample preparation techniques using a single instrument and controlling software. The sampler can be configured as part of a GC or LC system or can be configured as a bench-top workstation and can also include a six position evaporation station.

In this report, the automation of liquid-liquid extractions by the robotic autosampler is discussed. Examination of a new, automated vortexing option that allows samples to be rapidly and effectively mixed using speeds of up to 3000rpm is described. Automated liquid-liquid extractions methods for a variety of analytes from different matrices are examined and resulting precision and accuracy data are provided.

**Keywords:** Extraction, Laboratory Automation, Liquid Chromatography/Mass Spectroscopy, Sample Preparation

**Application Code:** General Interest

**Methodology Code:** Sampling and Sample Preparation
### Abstract

The last 15 years have shown an increasing requirements for the use of coatings to improve analytical results obtained in systems used for mercury and sulfur sampling. New flare monitoring regulations, sub part –Ja, go into effect in 2015 and require inert flow paths for low level sulfur analysis. New regulations for mercury emissions from coal fired boilers are now enacted and industry sampling initiatives for natural gas and oil wells drives a renewed need for inert sample paths. As a result of increased regulation, sampling system performance must be rugged and stable, even in challenging and corrosive environments, while also reliable to meet frequent calibration inertness checks. To meet stringent standards, the use of coatings has been specified or highly recommended.

This presentation will address and summarize reports to date on how coatings have been used to effectively improve the accuracy and reliability of sampling systems across many applications and industries. Industries and application data presented will range from flare gas and stack gas sampling to gas and oil exploration. All of these industries have unique challenges which must be addressed and solved by high durability, inert coatings that are designed for chemical compatibility.

### Keywords

- Adsorption, Process Control, Sulfur, Trace Analysis

### Application Code

- Regulatory

### Methodology Code

- Process Analytical Techniques
Pharmaceutical discovery research labs are a fast pace, high throughput environment where quality and efficiency are balanced for a fit-for-purpose approach. Typically, detailed lab work would need to be performed by skilled hands to ensure the accuracy and precision of standard curves and sample dilutions. This time consuming work is also a benchmark of quality in bioanalytical labs that only experienced staff can perform. We show that by using a bioprinter the quality and efficiency are increased while devoting time to other duties thereby increasing workflow productivity. The bioprinter performs the arduous tasks of preparing standard curves for bioanalysis and research compound dilutions for in vitro screening assays. While doing so with accuracy, reproducibility, relative ease, and speed. Utilization of a bioprinter has increased our DMPK lab productivity by 25% over manual labor and offers more possibilities that can be explored in the future.
## Abstract Text

Improving sensitivity and working with limited sample volumes without compromising the efficiency of the workflow is a challenge for many bioanalysts. In order to achieve the required detection limits many analytical methods utilize dry down and reconstitution steps to remove the dilution effects necessitated when using traditional scale SPE and operating with very low sample volumes.

Low bed weight SPE offers a potential solution to this problem by allowing users to take advantage of low elution volumes. As a result, dilution of low volume samples in the final eluent can be avoided. This means that sample usage can be reduced and issues caused by evaporation can be eliminated without compromising the sensitivity of the assay. Additionally larger sample volume can be used to achieve a concentration effect directly during the SPE process.

This poster presents a novel approach to low volume sample preparation. Reproducibility, robustness and ease of use at low elution volumes can be achieved by utilizing the revolutionary SOLA, Solid Phase Extraction (SPE) technology. This removes the need for frits delivering a robust, reproducible format which ensures highly consistent results at low elution volumes compared to traditional SPE and micro elution technologies.

The poster will demonstrate how this new approach will allow users to:

- Achieve a high level of confidence in analytical results at low elution volumes due to high reproducibility at low elution volumes
- Increase sensitivity by increasing sample loading and reducing elution volumes
- Improve productivity by removing requirement for lengthy evaporation and reconstitution
- Process samples which are limited in volume or compromised by evaporation procedure

### Keywords
- Biological Samples, Sample Preparation, Solid Phase Extraction

### Methodology Code
- Bioanalytical

### Application Code
- Sampling and Sample Preparation
Epilepsy affects approximately one percent of the world population. Of these patients, 70% experience local or focal seizures which occur in only one region of the brain. Epileptic patients are diagnosed after having two or more non-provoked seizures. By giving an animal multiple seizures within one experiment, the model more accurately represents epileptic patients, improving on the animal model for local epilepsy. Additionally, epilepsy is believed to result in oxidative stress in the body. By monitoring oxidative stress biomarkers as well as neurotransmitters, we hope to gain a better understanding of how epilepsy correlates to oxidative stress. In this study, microdialysis was used to locally dose the hippocampus of a rat with an epileptic agent, 3-mercaptopropionic acid (3-MPA), while simultaneously collecting extracellular fluid for analysis. Samples were analyzed for the excitatory and inhibitory responders, glutamate and GABA, via liquid chromatography coupled to fluorescence detection. Changes in these amino acids indicate that a seizure occurred and give information on seizure strength. Catecholamine neurotransmitters norepinephrine and dopamine were also monitored using liquid chromatography coupled to electrochemical detection. Monitoring the both amino acid and catecholamine neurotransmitters gives additional neuronal information about epilepsy. Biomarkers of oxidative stress such as malondialdehyde were also monitored showing the oxidative effects of epilepsy. Various time regimens were explored in order to study seizure strength and duration in response to the epileptic agent. It was determined that there was approximately a twofold decrease in glutamate response to a secondary epileptic episode. It is possible that desensitization to the epileptic agent is occurring which may be indicative of glutamate depletion, a neuronal protective response, or changes in neuronal plasticity which could be caused by oxidative stress.

Keywords: Bioanalytical, Capillary Electrophoresis, Liquid Chromatography, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Sampling and Sample Preparation
EPA Method 526 is used to determine selected water-soluble, semi-volatile pesticides, herbicides and additives in raw and finished drinking waters. One liter water samples are passed through polystyrene-divinylbenzene solid phase extraction (SPE) cartridges and the analytes of interest are recovered with ethyl acetate and dichloromethane elutions of the dried sorbent bed. The sample are then concentrated and analyzed by gas chromatography/mass spectroscopy (GC/MS).

The PrepLinc™ Large Volume Injection (LVi) system allows the user to pass large volumes of aqueous sample matrix through commercially available SPE cartridges and disks. The autosampler will accommodate 1 liter sample jars, therefore sample is taken directly from the container used to collect the water in the field. The analytes of interest can be eluted to collection tubes or to an AccuVap module for concentration directly to autosampler vials. In this study, the labor intensive steps of SPE conditioning, loading, elution and concentration were reduced to two steps: 1) loading on to the instrument and 2) programming the sequence with saved method parameters. This provided for a fast and simple automated method for the labor intensive process of manually loading 1 liter of water to an SPE, subsequent elution and concentration for analysis.

Keywords: Automation, Environmental Analysis, Solid Phase Extraction, Water
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Sampling and Sample Preparation Techniques

High-Throughput Automated Analytical Platform for E. coli Derived Antibodies

Escherichia coli (E. coli) is a non-traditional platform for producing antibodies that offers potentially higher production yield and rapid fermentation process development compared with its counterpart Chinese Hamster Ovary cells (CHO). However, the expression platform comes with its own challenges such as the requirement of cell lysis for product retrieval due to the lack of protein secretion and often minimal folding fidelity, necessitating refolding procedures. Because of these factors, the analytics associated with the development of E. coli derived antibodies can be challenging, especially for expression screening and fermentation optimization. Here we describe an automated platform developed and established for the rapid analysis of whole cell broth. This includes the initial lysis of the cells, analysis of the resulting yield, downstream affinity purification, and further analytics such as SEC and icIEF. We have developed a high throughput platform with an integration of automation, information and analytical technologies. The platform is able to produce fast data enabling just-in-time decision making for process development.

Keywords: Chromatography, High Throughput Chemical Analysis, Process Analytical Chemistry, Sample Handling
Application Code: Pharmaceutical
Methodology Code: Sampling and Sample Preparation
This study explores the stability of veterinary drug target analytes, from several compound classes in a variety of tissues stored at different temperatures under a range of conditions. Despite common understanding of storage condition requirements, e.g., -80°C vs. -20°C, there are many situations where sample temperature control is not adequate, potentially placing the sample integrity in question. Given the number of variables required to optimize LC-ESI-MS/MS for multi-residue/multi-class analyses, including matrix considerations from the bench to the instrument, it is important not to overlook the quality of the sample following storage prior to the analytical procedure.

This work was initiated following the observation that coccidiostats spiked into turkey liver were differentially affected during storage at temperatures of -20°C and -80°C, where the greatest loss of analyte was observed at -20°C. This is evidenced in the accompanying figure which shows overlaid chromatograms for monensin extracted from turkey liver which had been freshly spiked at 50ppb (control), and samples which had been spiked at 50ppb and then stored at -20°C and -80°C respectively. The response for the sample stored at -20°C was about 30% as compared to the control and the sample stored at -80°C.

These findings inspired an in depth evaluation of sample storage conditions required for regulatory samples and tissues to be used in method development. Evaluation was carried out with design of experiment (DOE), including fractional factorial with ANOVA for variables such as sample size, spike or incurred analyte concentration, storage time, temperature, tissue type, and species such as turkey and chicken.

Keywords: Drugs, Sample Preparation, Sampling, Temperature
Application Code: Regulatory
Methodology Code: Sampling and Sample Preparation
Sampling and Sample Preparation Techniques

Using SPE to Achieve a Twenty Fold Pre-Concentration without the Need for Evaporation and Reconstitution

Despite advances in analytical detection technology, achieving required limits of assay sensitivity can still be challenging for many bioanalysts. In order to improve limits of detection analysts are looking to sample preparation not only for increased sample cleanliness but also to pre-concentrate their sample prior to analysis.

Traditional scale SPE facilitates clean up of the sample to minimize matrix effects, however in order to pre-concentrate the sample a lengthy dry down and reconstitution step needs to be employed. This process is not only time consuming but can have a detrimental effect on the recovery of the analyte often due to volatility or non specific binding.

This poster presents a low elution volume sample preparation protocol for the analysis of niflumic acid in human plasma, which allows users to achieve a twenty fold pre-concentration without the need for evaporation and reconstitution. The results demonstrate that this level of sample pre-concentration can be achieved without sacrificing reproducibility, robustness and ease of use.

Keywords: Biological Samples, Sample Preparation, Solid Phase Extraction
Application Code: Bioanalytical
Methodology Code: Sampling and Sample Preparation
Sampling and Sample Preparation Techniques

Use of Unique Retention Properties of Graphitized Carbon Toward Passive Sampling of 1,3-Butadiene and Other Hazardous Pollutants in Air

Proposed EPA Method 325 uses the technique of passive sampling to collect air samples along the fence line of petroleum refineries. The target compounds include Hazardous Air Pollutants (HAP) such as; 1,3-Butadiene, Benzene, Toluene, and Xylenes. The samples are analyzed using Thermal Desorption and Gas Chromatography. The proposed method provides an economical way of determining the annual average concentrations of VOC’s emitted from the refinery along its perimeter.

The passive air sampling device used in this method is comprised of a stainless steel thermal desorption tube packed with a single bed of graphitized carbon. The inner surface of the stainless steel tube is specially treated with a protective inert layer to mask the active sites which could be present on the inner surface of the tube.

In this work we evaluated a number of different adsorbents for their background levels of target compounds, including benzene. We tested the adsorbent’s ability to retain a broad list of compounds under dry and humidified conditions. The chosen graphitized carbon has the unique capabilities to retain all analytes.

Keywords: Environmental Analysis, Monitoring, Sampling, Thermal Desorption
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation

Primary Author: Jamie L. Brown
Co-Author(s): Daniel Vitkuske, Kristen Schultz, Michael Ye, Olga I. Shimelis
Pharmaceuticals and Personal Care Products (PPCPs) have been found in many surface water systems throughout the world. Concentrations are typically reported in the ng/L range so sample preparation and preconcentration are required for detection. Manual off-line solid phase extraction (SPE) is one method for sample preparation and is specified in EPA Method 1694. Though up to 1L of sample can be extracted in this way, the manual aspect requires a great deal of operator time and several hours of laboratory time. On-line SPE has also been developed using a trapping column ahead of the analytical column. The alternative developed here involves using a valve block system with multiple semi-preparative columns for automated parallel SPE. The extract is then evaporated to reduce volume for analysis. Results from this method using a Semba Biosciences Octave 100 system to prepare 1L samples for analysis of PPCPs by LC-MS/MS will be presented.
Analysis of Volatiles Organic Compounds (VOC) in water is a growing field of interest, due to the need of a continuous confirmation of the quality of drinking water and the control of the contaminants in waste water.

The Environmental Protection Agency (EPA) strictly regulates this kind of analysis through the method 8260 in which detection limits and instrumentation requirements are established.

This work presents the GC analysis of VOC in water, in compliance with EPA method 8260, obtained with the use of Purge and Trap coupled with TOF-MS. The DHS-P&T guarantees the complete VOC extraction from water without any sample carryover or cross contamination and the TOF-MS allows to reach the Minimum Detection Limit required working with extended mass range. Moreover, its high acquisition rate, coupled with a new approach to deconvolution, provides a proper compound identification even when compounds are co-eluted, assuring a proper identification of any VOC present.

In addition, the possibility to overlap sample incubation in the DHS-P&T and to perform Fast GC, results in an increased laboratory productivity.

The results obtained show that the Purge & Trap technique provides the sensitivity needed to exceed the low-level threshold required by the latest regulations. Data will be reported including chromatographic parameters, calibrations and method detection limits.
Extraction of Poly Aromatic Hydrocarbon (PAH) compounds from water involves using a large volume of solvent. The advent of more sensitive Mass Spectrometers (MS) coupled with Large Volume Injection (LVI) onto the Gas Chromatograph (GC) has aided in better detection of PAH compounds. In consequence, micro-extraction of PAH compounds from water has become a viable solution for sample preparation. Micro-extraction reduces sample size, and solvent usage, thus decreasing laboratory costs and sample preparation time. This application will investigate an automated liquid-liquid extraction technique for the preparation of PAH water samples.
In an effort to improve product performance and/or make products safer, certain compounds are added. Phthalates, used to make plastic more pliable, and flame-retardants, are two prime examples. Unfortunately, some of these are environmentally hazardous, and therefore toxic to humans, so require monitoring. Certain phthalates are banned in children’s toys and child care articles in the US; and brominated flame retardants, widely found in indoor and outdoor environments, and shown to have adverse health effects are also banned in the US and EU. Other newly developed additives may yet have unknown health risks. Even residual components used to make a polymer (like styrene from polystyrene and bisphenol A from polycarbonate) may be left on the polymer or leach from the polymer with aging and wear. Analysis often of requires lengthy sample preparations, like solvent extraction. The solvent itself becomes a problem, as it may also be environmentally hazardous, and requires proper disposal.

A simpler way to analyze such compounds would be thermal extraction. Requiring no solvents, a sample is simply heated. At lower temperatures (like 300°C) semi-volatiles, such as phthalates and other additives are removed from the product in question, transferred directly to the gas chromatograph. At higher temperatures, like 750°C or greater, nonvolatile molecules, like polymers or larger additives, can be broken down into volatile fragments which, in turn, can also be analyzed using a gas chromatograph. In this presentation, consumer products will analyzed for potentially toxic compounds like phthalates and flame-retardants using thermal extraction techniques. Both thermal desorption and pyrolysis will be used to study these known toxic and potentially toxic compounds.

Keywords: GC-MS, Pyrolysis, Sample Preparation, Thermal Desorption

Application Code: Environmental

Methodology Code: Sampling and Sample Preparation
The Environmental Protection Agency has proposed new legislation for US petroleum refineries to control emissions from storage tanks, flares and coking units. When fully implemented, the rule will result in an estimated reduction of 5600 tons per year of toxic air pollutants and 52,000 tons per year of VOCs, improving air quality and protecting public health for workers and surrounding communities. Integral to the new law will be the requirement to monitor air concentrations of benzene at the perimeter fence-line. Diffusive monitoring has been widely used in a range of air monitoring scenarios, i.e. occupational hygiene, as well as plus indoor air and ambient air monitoring. By eliminating the requirement for a sampling pump, diffusive monitoring provides a simple and cost-effective method of collecting the large number of samples required in many air monitoring programmes. Key applications include personal exposure monitoring, large-scale environmental studies, and indoor air monitoring. Widespread use over many years has resulted in over 100 published uptake rates (e.g. ISO-16017 part 2). This simplifies matters for new users, as often uptake rates are available without the need to determine them experimentally.

This poster will discuss the application of passive sampling with industry-standard sorbent tubes and factors that need to be taken in to account when deploying them.
Pseudomonas aeruginosa is one of the leading causes of gram-negative bacterial infections in hospital settings. P. aeruginosa’s antibiotic-resistant character has results in great concern within the scientific community. Given its prevalence and impact on people with compromised immune systems and cystic fibrosis, developing a novel means of detecting P. aeruginosa is a top priority. One such method targets P. aeruginosa’s production of pyocyanin, a quorum sensing molecule (QSM) that is unique to this species. As pyocyanin is redox-active, it can be detected electrochemically using standard electrochemical techniques, such as differential pulse voltammetry (DPV), where the sensitivity of the measurements is limited by the concentration of pyocyanin present in the sample. Use of up-regulatory molecules to increase P. aeruginosa’s production of pyocyanin has yet to be utilized for early and rapid detection in complex samples. Therefore, the aim of this study is to electrochemically detect and optimize the up-regulation of pyocyanin production, leading to a better understanding of how this bacterium grows at the onset of infection as well as improving this sensing strategy.

Different amino acids, iron compounds, metal halide salts, and simple sugars were explored as a means of regulating P. aeruginosa cell growth. Cultures of P. aeruginosa were grown overnight and inoculated into separate liquid media. Samples were taken at set time intervals, loaded onto a disposable, carbon-based screen-printed electrode, and DPV was performed to determine the concentration of pyocyanin. The data from this study provides a better understanding of how pyocyanin production is linked to the up-regulation of biofilm formation in P. aeruginosa and supports the use of electrochemical techniques for early infection identification. This approach can be extended to other pathogens by identifying and regulating other unique QSMs.

This work was supported by the NSF under grant no. 1125535.
Reference electrodes are used in almost any electrochemical measurement. In many reference electrodes, the electrolyte solution that forms a salt bridge between the sample and the reference electrode are contained in nanoporous glass plugs (such as Vycor or CoralPor glass). Although these reference electrodes are frequently used, their use is limited to solutions with high ionic strength, because at low ionic strength, the half-cell potentials of reference electrodes are not sample independent and depend on the sample composition. The variations in the reference potential cannot be explained by the liquid junction potential between two miscible electrolyte solutions, and they result from the surface charge density on the glass surface and the resulting electrostatic screening of ion transfer into the glass pores when the dimensions of the pores are comparable to or smaller than the Debye length. To overcome this issue, nanoporous polymeric materials that bear no surface charge were synthesized and the performance of the reference electrodes equipped with these plugs were tested. Porous polystyrene with average pore diameter of 10.0 nm was shown to be a proper material for construction of reference electrodes for organic solvents. Poly(ethylene oxide)-block-poly(styrene-co-divinylbenzene), which is more hydrophilic compared to polystyrene, is synthesized for use as plugs in reference electrode for aqueous solutions.

Crosslinked Highly Fluorinated Polymers for Ion-Selective Electrodes

It has been previously shown that fluorous membrane based ion-selective electrodes (ISEs) have improved selectivity and an improved resistance to biofouling compared to those with conventional plasticized polymeric membranes, such as polyvinyl chloride. Most previous fluorous membrane ISEs have relied on a fluorous liquid deposited into a porous poly(1,1,2,2-tetrafluoroethylene) support. This has the advantage that any type of fluorous liquid can be tested readily as ISE membrane matrix but it limits the mechanical stability of these sensors and the ability to miniaturize them. One previously introduced approach to address this problem was the introduction of plasticized poly[4,5-difluoro-2,2-bis(trifluoromethyl)-1,3-dioxole]-co-poly(tetrafluoroethylene) as the ion-selective membrane matrix [1]. To test the possibility of eliminating the plasticizer and the need for solvents in the device fabrication, we have recently shown highly fluorinated polymers with low glass transition temperature, such as poly[(1H,1H,2H,2H-perfluorooctan-1-yl)styrene], can function as ion-exchange membranes with a wide selectivity range similar to that of ion-exchange membranes based on fluorous liquids and as highly selective silver ISEs. However, these electrodes still required a porous support. In this work, we present ion-exchanger electrodes and silver ISEs that have been fabricated using self supported membranes of crosslinked highly fluorinated polymers. These electrodes show a Nernstian response, and show a wide selectivity range or are highly selective for silver. Theses sensors show improved mechanical stability and an ability to be miniaturized compared to that of previous fluorous membrane ISEs.


Keywords: Electrochemistry, Electrodes, Ion Selective Electrodes, Polymers & Plastics
Application Code: Other
Methodology Code: Electrochemistry
Polycyclic aromatic hydrocarbons 9,10-diphenylanthracene (DPA) and rubrene (RUB), along with tris(2,2'-bipyridine) ruthenium (II) species [Ru(bpy)$_3$$^{2+}$], have shown excellent electrogenerated chemiluminescence (ECL) at ~410, 550, and 620 nm, respectively. Their use as ECL labels for multiplexing detection of biomolecules, however, is limited, as both DPA and RUB are very insoluble in water. We report herein the electrochemical and ECL studies of individual sulfonated DPA and RUB (synthesized in our lab) and Ru(bpy)$_3$$^{2+}$ as well as their mixtures in aqueous media. Data obtained from various anodic and cathodic ECL coreactants including 2-(dibutylamino) ethanol, tri-$i$-propylamine, persulfate, and hydrogen peroxide at different working electrodes (glassy carbon, Pt, and Au) were compared. Correlation between the individual ECL emitter’s concentration and its ECL peak intensity from the mixed systems under optimal experimental conditions (i.e., type and concentration of the coreactant, type of the working electrode etc.) was established. This study provides us the basis of ECL based multiplexing detection of biomolecules in aqueous media.

Financial support from the NSF CAREER Award (CHE-0955878) is gratefully acknowledged.

Keywords: Chemiluminescence, Electrochemistry, Fluorescence, Voltammetry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
The novel structurized - layered potentiometric potassium- or copper-selective photopolimerized polyacrylate membranes (containing the same contents of ionophore and ion-exchanger, in each layer) were studied. Thus a traditional single membrane characterized by one diffusion coefficient can be replaced by ordered structure of a few layers with different diffusion coefficients. It was shown that this approach allows to control the overall primary ion diffusion rate in the membrane. The ultimate result is slowing down the full saturation of the membrane phase with primary ions. In this way it was possible to obtain improved potentiometric responses of the tested sensors.
Electron Transfer Kinetics of Hydrazine Oxidation on Single Nanoparticles

Metal nanoparticles of various size, shape and composition are the catalysts of choice for a wide range of energy technology applications. Typically, measurements made from nanoparticle catalysts are the averaged responses from an immobilized ensemble on an electrode and can depend on the spatial organization and the number of particles present. Understanding what effect size and shape have on the intrinsic catalytic activities of individual nanoparticles is fundamentally important, yet remains a significant challenge.

Using the technique of Fast Scan Cyclic Voltammetry (FSCV) we are able to measure the specific electrocatalytic activities of individual metal nanoparticles for the fuel cell relevant Hydrazine Oxidation Reaction (HOR). FSCV enables us to measure many Cyclic Voltammograms (CVs) from each nanoparticle, as they collide with an inert electrode, and are used to determine the electrocatalytic activity and Electron Transfer (ET) kinetics. We observed the oxidation of the HOR shifts to more positive potentials as the particle size decreases, possibly due to stronger surface binding of the nanoparticle ligands and the increasing mass transfer rate of reactant, requiring a higher potential to overcome these effects. From the ET kinetics, the smallest sized nanoparticles were found to possess the largest value for the heterogeneous electron transfer coefficient which was used to calculate the Gibbs free energy of activation. We found the change in Gibbs free energy follows a thermodynamic model where it is dependent on the radius of the nanoparticle and can be ~11.8 kJ/mol smaller than the bulk metals.

Keywords: Electrochemistry, Nanotechnology, Voltammetry
Application Code: Nanotechnology
Methodology Code: Electrochemistry
Over the past decade, several reports have emerged describing collisions of different types of particles (i.e., metals, oxides, polymers, emulsion droplets, and liposomes) on ultramicroelectrodes. The collision frequency and shape/size of the collision events can yield insight into concentration, shape, and size distribution of the specific colloid. A method using electrochemical collision techniques for the specific determination of the murine cytomegalovirus will be presented, and future directions will be discussed.
Synthesis and Analysis of PdPt Nanoalloys via Alloying Individual Bulk Pd and Pt Metals in Molten Lithium for Methanol Electro-Oxidation Applications

Heterogeneous catalysts are often optimized by substituting monometallic nanoparticles with multimetallic alloys for their enhanced stability and selectivity. Current state of the art nanoalloy synthesis techniques involve the chemical reduction of metal ions in a solvent containing organic surfactants or stabilizing agents, which prevent aggregation of the resulting nanoparticles. Chemical reduction synthesis may lead to unintended core-shell formation, agglomeration of nanomaterials, or support degradation upon removal of stabilizing agent.

Herein we report a complimentary approach to synthesizing metal nanoalloys directly from individual bulk metals by dissolving them in molten lithium; avoiding the use of stabilizing agents, metal salts, or bulk metal alloys. Two systems will be discussed. First, palladium-platinum (PdPt) was chosen for Pd and Pt’s similar crystalline structure (face-centered-cubic, atomic radius of 137 and 139 pm, respectively). This was then expanded to include metals with dissimilar structure such as PdZn in which zinc displays hexagonal close-packed crystalline structure with an atomic radius of 134 pm. Alloy formation was confirmed and insights into the mechanism of nanoalloy synthesis were explored using multiple x-ray techniques including powder x-ray diffraction (PXRD), extended x-ray absorption fine structure (EXAFS), and x-ray absorption near edge structure (XANES). Electrochemical measurements were performed to investigate the characteristics of as-prepared PdPt nanoalloys as well as the catalytic activity towards methanol electro-oxidation. Electrochemical studies show that the PdPt nanoalloys exhibit a 72.0% increase in specific activity for the methanol oxidation reaction when compared to as-prepared Pt nanoparticles.

Keywords: Electrochemistry, Materials Characterization, Nanotechnology, X-ray Diffraction
Application Code: Fuels, Energy and Petrochemical
Methodology Code: X-ray Techniques
The development of better catalysts for oxygen reduction reaction (ORR) and other electrocatalytic processes requires detailed knowledge of reaction pathways and intermediate species. Here we report new methodology for detecting charged reactive intermediates and its application to mechanistic analysis of ORR. A nanopipette filled with organic phase immiscible with the external aqueous solution was used as a tip in the scanning electrochemical microscope (SECM) to deliver neutral redox species (O2) to the electrocatalytic surface. The transfer of a negatively charged intermediate of ORR (superoxide, O2-) to the organic solution produced ion transfer (IT) current across the liquid/liquid interface. The time required for an ion to cross the nanometer-thick gap between the catalytic surface and the pipette orifice was <1 s—sufficiently short to detect and quantitate O2- and other short-lived intermediates. The detection is highly selective because the IT current is insensitive to neutral molecules, and different ionic species can be distinguished based on their charge and standard IT potential. The developed approach is suitable for mechanistic studies of numerous electrocatalytic processes and other heterogeneous reactions involving ionic intermediates.

**Keywords:** Detection, Electrochemistry, Electrode Surfaces

**Application Code:** Fuels, Energy and Petrochemical

**Methodology Code:** Electrochemistry
Electrochemical biosensors have been explored as a novel approach for DNA detection because of their portability, low cost, and high sensitivity. Here, we present a four-way junction electrochemical sensor developed using screen printed gold electrodes. The sensor is comprised of a stem loop oligonucleotide (SL) immobilized via a gold-thiol bond to the electrode surface and two synthetic adaptor oligonucleotide strands used for hybridization to target analyte. The adaptor strands, one labeled with methylene blue, have a segment complimentary to the SL and to the analyte. A four-way junction is preferred over a two strand DNA detection system due to its improved selectivity and the potential for using one universal optimized reporter for detection of any DNA or RNA sequences.\[1\] Square Wave Voltammetry (SWV) was used to detect hybridization of target microRNA (miRNA) by analyzing an increase in current caused by electron transfer between the methylene blue and the electrode surface. No electrochemical signal was observed in the absence of the target miRNA strand. The sensor has been optimized to produce maximum signal intensity using 0.1 M solution of the SL, producing a surface probe density of 5.78 x 10\[11\] molecules/cm\[2\]. Altering the sequence of one segment on each adaptor strand allows this electrochemical sensor to be used for a variety of target analytes, offering the possibility for a universal sensor format.

The authors acknowledge the College of Sciences and the Department of Chemistry at the University of Central Florida for financial support of this research.

A novel scheme for the spatially-resolved detection of lithium ions in aqueous solutions was developed. We utilize cyclohexane for the facilitated ion transfer of lithium ions across the water/(1,2-dichlorethane + cyclohexane) interface supported at laser pulled micro- and nano-quartz pipettes. This ITIES system is highly sensitive and produces steady-state electrochemical signals that depend on the local concentration of lithium ions.

The ITIES pipettes are used as probes for high resolution scanning electrochemical microscopy (SECM) imaging of active TiO\(_2\) substrates, which are known to insert lithium ions when biased at reducing potentials. SECM imaging with ITIES pipettes is able to discern site-specific surface phenomenon and demonstrate heterogeneity in the electrochemical performance of TiO\(_2\) substrate electrodes for insertion of lithium ions in real-time. The insertion efficiency for lithium is known to vary based upon the crystalline phase of the TiO\(_2\) \cite{1}. ITIES-SECM imaging is used to study the spatial diversity in local ionic concentrations for lithium as a function of the crystal structure of TiO\(_2\) and electrode potential. The images produced by ITIES-SECM are corroborated by optical microscopy and Raman imaging for identification of electrochemically active structures and regions. The Raman and optical microscopy has been reported previously \cite{2}.

ITIES-SECM has been shown to be a highly promising platform for lithium ion detection and high spatial resolution imaging of energy materials for evaluation of their electrical storage performance.

References:

Keywords: Electrochemistry, Materials Characterization, Surface Analysis, Voltammetry
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Electrochemistry
Ion transfer at the interface between two immiscible electrolyte solutions (ITIES) allows for the detection of non-electrochemically redox active species using traditional electrochemical techniques. This can be especially useful in the detection of neurotransmitters such as acetylcholine, which is difficult to be detected directly using a traditionally used carbon electrode, where the modification of the electrode surface is often required. In this work, quartz capillary pipettes were laser pulled to form a pore on the nanometer scale, and were filled with 1,2-dichloroethane to create an ITIES interface when immersed in artificial sea water. We were able to detect several neurotransmitters both qualitatively and quantitatively at the nano-ITIES. Additionally, we investigated the transfer behavior of these neurotransmitters and compared these findings to those at larger interfaces. The steady state current obtained by the transfer of the neurotransmitters allowed for their quantitation at physiological conditions relevant to our model animal, [i]Aplysia californica[/i]. Furthermore, the small size of these nanopipettes means that they may be used as amperometric probes for the high resolution imaging of the neurotransmission process using scanning electrochemical microscopy.

Acknowledgement: This research was supported by the National Institutes of Health under Award Number R21NS085665.

Keywords: Electrochemistry, Nanotechnology, Neurochemistry, Quantitative
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Ion Selective Electrodes (ISEs) are widely used to determine the concentration of a specific ion dissolved in aqueous solution. The goal of this project is to design a carbonate ISE in which a perfluoroalkyl ketone serves as the ionophore in a fluorous liquid phase membrane. Perfluoroalkyl ketones are potential ionophores for the doping of membranes of ion selective electrodes due to the proposed superiority of binding with carbonate ions. NMR dilution and titration experiments were used to estimate the binding constant between an analogous ionophore and carbonate. Trifluoroacetophenone (TFAP) was used as a suitable analogue of perfluoroalkyl phenyl ketones because of the limited quantity of perfluoroalkyl ketone ionophore available. Cryptand-2,2,2 was applied in this system because we employed potassium carbonate as the carbonate source and because it strongly binds potassium cations and thereby minimizes ion pairing with carbonate. In the NMR dilution experiment, a series of NMR samples of TFAP, K\(_2\)CO\(_3\) and cryptand in deuterated methanol were diluted with the TFAP: carbonate ratio of 1:2, 1:1 or 2:1. A NMR titration experiment was performed holding the concentration of host constant while varying the concentration of guest. The complex formation was further studied by Job plots and species plots. Non-linear regression was carried out using Mathematica 9.0. From the NMR dilution experiments, the logarithm of the binding constant for a 1:1 guest to host ratio was calculated to be 2.77 ± 0.03 while for a 2:1 complex, the binding constant was 1.9 ± 0.2. So far, from the data of the dilution NMR experiment, we can conclude that binding of the potassium ion with cryptand-2,2,2 increases the stability of the complexes between carbonate and the perfluoroalkyl phenyl ketone ionophore.

Keywords: Ion Selective Electrodes, NMR, Potentiometry, Sensors
Application Code: Clinical/Toxicology
Methodology Code: Magnetic Resonance
Trifluoroacetophenone derivatives have been known to selectively bind to carbonate through covalent bond formation and are commonly used as ionophores in carbonate ion selective electrodes. Heptadecafluorooctyl phenyl ketone was previously synthesized as a new ionophore designed to selectively bind to carbonate. Synthesizing a carbonate compound lipophilic enough to determine this new ionophore’s affinity for carbonate by way of a NMR titration experiment, however, was unsuccessful. Instead, potassium carbonate was tested to determine its suitability for determining carbonate binding. Spectroscopic methods were used to study the interaction between carbonate and a widely-available, fluorophilic analog to heptadecafluorooctyl phenyl ketone, trifluoroacetophenone. NMR dilutions, titrations, and a Job plot coupled with original Mathematica programs specifically designed to simultaneously fit data from multiple experiments provided binding constants for both 1:1 and 2:1 trifluoroacetophenone-carbonate complexes as well as chemical shift values for each complex formed. In addition, for the NMR dilution experiments, the binding constant results made it possible to prepare speciation plots describing the concentrations of free ionophore and each complex formed throughout the course of the dilution experiments. Results indicate weak binding between trifluoroacetophenone and carbonate with 1:1 and 2:1 log scale binding constants of 1.70 ± 0.15 and 1.59 ± 0.35, respectively. From these results, using a Mathematica file to concurrently fit multiple NMR experiments produced reliable binding constants for the trifluoroacetophenone-carbonate complex, confirming both 1:1 and 2:1 ionophore-carbonate complex formation. We are planning to apply this optimized method to the new fluorophilic carbonate receptor, heptadecafluorooctyl phenyl ketone.
**Session Title**
SEAC - Society for Electroanalytical Chemistry Poster Session

**Abstract Title**
All-Solid-State Ion-Selective Electrodes and Reference Electrodes Based on Colloid-Imprinted Mesoporous Carbon

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## Abstract Text
All-solid-state ion-selective electrodes and reference electrodes are miniaturized electrochemical sensors with an intermediate layer between a sensing membrane and an underlying solid electron conductor. In this work, colloid-imprinted mesoporous (CIM) carbon with a low content of redox impurities and high double layer capacitance is investigated as intermediate layer to construct all-solid-state ion-selective electrodes and reference electrodes. These sensors exhibit superior electrochemical performance including excellent ionic response, excellent resistance to common interfering agents such as O₂, CO₂, light and water, as well as outstanding potential stability. When combined with a redox couple as internal reference species, calibration-free all-solid-state ion-selective electrodes and reference electrodes can be fabricated. In view of mass production, the synthesis of CIM carbon and fabrication of the corresponding sensors are straightforward and employ inexpensive precursors, making CIM carbon a promising component for commercialized all-solid-state electrochemical sensors.

**Keywords:**
Electrochemistry, Ion Selective Electrodes, Nanotechnology, Sensors

**Application Code:**
Clinical/Toxicology

**Methodology Code:**
Electrochemistry
Despite the importance of the second dimension separation in determining the overall performance of two-dimensional liquid chromatography (2D-LC) separations, the characteristics of the second dimension separation bear little resemblance to what most would consider mainstream liquid chromatography. Second dimension separations must be faster and more repeatable (in absolute time units) than what is typically required of conventional 1D separations. From a theoretical point of view, these requirements push us to the edges of both understanding and practice – we want to use short narrow columns, packed with small particles, and operated at high pressures and temperatures, and very high eluent linear velocities; at the same time we want to inject hundreds of fractions of first dimension effluent with volumes on the order of the column volume per hour.

In this presentation we will share some of our observations made when dealing with this chromatographic quagmire. Specifically, we will discuss the impact of the demanding conditions associated with the second dimension on column lifetime, and steps that can be taken to slow the deterioration of column performance to a practically tolerable level. We will also discuss the impact of the first dimension effluent composition on the performance of the second dimension, both for non-ionizable and ionizable solutes in terms of detection sensitivity and choices related to column selection and method development.

Keywords: Bioanalytical, Environmental, Food Contaminants, Liquid Chromatography
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
A good deal of progress has been made in developing the instrumentation to do fast, two-dimensional chromatography (2D-LC) and understanding the factors needed to optimize its resolving power. Although there have been a few notable experimental and theoretical studies of peak quantitation we do not fully understand, as yet, why the reproducibility of the peak size in 2D-LC is poorer, and sometimes considerably poorer, than in 1D-LC. Some recent results that clarify the source of this problem will be presented.

There is a great deal of unfulfilled potential inherent in the higher order data structure of two-dimensional liquid chromatography when done with a multi-variate detector. Methods such as parallel factor analysis (PARAFAC), the generalized rank annihilation method (GRAM) and multivariate curve resolution-alternating least squares (MCR-ALS) approaches possess many advantages relative to conventional quantitation methods. These will be discussed here.

Keywords: Data Analysis, Liquid Chromatography, Liquid Chromatography/Mass Spectroscopy, Quantitative
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
The use of elevated column temperature has long been viewed as a practical solution to achieving greater separation speed. While increased column temperature reduces eluent viscosity and increases analyte diffusivity, both attributes that enhance separation quality, higher column temperature often requires a commensurate reduction in the elution strength to maintain solute retention. Thus, high-temperature reversed phase separations are frequently performed using nearly aqueous mobile phases. This presents a major problem for fast, high sensitivity measurements of modestly hydrophobic solutes in capillary liquid chromatography: inadequate preconcentration. Large volume injections of aqueous samples onto high efficiency capillary columns operated with nearly aqueous eluents results in volume overload-induced losses in column efficiency. We are developing temperature-assisted on-column solute focusing (TASF) to address this problem. TASF mitigates precolumn dispersion by cooling a small segment of the column head to sub-ambient temperatures. Following the injection, the focusing segment is rapidly heated to match the temperature of the analytical segment. The use of active temperature control to enhance preconcentration and decrease volume overload effects has proven effective in a series of applications: 1) rapid separations of moderate and high polarity small molecules and peptides, 2) augment the typically used solvent-based focusing method and 3) facilitating the use of large volume injections of strong elution strength samples.

**Keywords:** Chromatography, High Temperature, HPLC, HPLC Columns

**Application Code:** Bioanalytical

**Methodology Code:** Liquid Chromatography
Once a good comprehensive two-dimensional liquid chromatography (LC×LC) method has been developed it may offer great advantages. Structured chromatograms may be obtained for very complex, non-volatile mixtures with potentially high peak capacities in reasonable analysis times. LC×LC systems are hardly more complex than common one-dimensional LC systems and they exhibit a similar high robustness.

It is generally true that the development of chromatographic methods requires more knowledge and skills than their application. Developing, improving and optimizing LC×LC methods requires amplified skills, as indicated below.

1. The analyst needs to select and (partly) develop two one-dimensional methods that offer very different (“orthogonal”) selectivities;
2. The individual separations stages need to be optimized within the constraints of time and pressure, as well as possible constraints of the sample and the detection system;
3. Losses in resolution (peak capacity) due to first-dimension “undersampling”, second-dimension band broadening and incompatibility of the first-dimension effluent with the second-dimension system need to be minimized, as does the dilution of the sample during the two separation stages;
4. The operating parameters in both dimensions must be optimized as a function of time (implying that the LC×LC is more than the product of two one-dimensional LC methods).

To help chromatographers make full use of the fantastic possibilities of LC×LC we are developing efficient, user friendly strategies and software tools. In this lecture the development of successful LC×LC methods will be illustrated with practical examples.

Keywords: HPLC, Optimization, Other Hyphenated Techniques, Polymers & Plastics
Application Code: Polymers and Plastics
Methodology Code: Separation Sciences
Abstract Text

Over the last decade the pharmaceutical industry has been inundated with ultrahigh pressure liquid chromatography (UHPLC) and all of the speed, efficiency and cost savings it theoretically affords. While powerful for many applications, a decade after its introduction UHPLC is still in a staggered phase of implementation within many pharmaceutical companies. Laboratories running standardized assays have experienced a higher uptake than development and quality control laboratories, where integration of UHPLC must be balanced with internal/external resourcing capabilities and regulatory requirements for method transfer/validation. Nevertheless, the impact that UHPLC has had on pharmaceutical method development cannot be ignored. In addition to hundreds of new HPLC columns, UHPLC has catapulted technologies such as 2D HPLC and online HPLC to go from customized lab-built systems to “off the shelf” analytical tools.

In this presentation, we will present our experiences applying UHPLC method development strategies and the challenges we have encountered installing them in various phases of development. We will also discuss technologies enabled by UHPLC that we have implemented to expand upon our current capabilities, focusing on decreasing the time to turn data into information enabling decision making.

Keywords: HPLC, Liquid Chromatography, NMR, Sample & Data Management
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
W. W. Coblentz, during his post-doctoral studies at Cornell University, and at the Bureau of Standards in the early 1900’s, did the basic studies that defined the analytical applications of infrared spectroscopy. The data he produced using instruments he constructed are remarkably good.

The Coblentz Society was founded in 1953 “To foster the understanding and applications of infrared spectroscopy”. As an example of the Society programs, high quality reference spectra have been generated in accordance with specifications established by the Society. These specifications are currently undergoing revision to take into account the current state of modern instrumentation. The Society supports training courses. It also supports symposia at national meetings to recognize developments in the field and presents a number of awards to leaders in the field. Current programs will be briefly described.

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Keywords: Analysis, Infrared and Raman, Molecular Spectroscopy, Vibrational Spectroscopy
Application Code: General Interest
Methodology Code: Molecular Spectroscopy
Probing Liquid/Solid Interface Chemistry Within Porous Particles by Confocal Raman Microscopy

Practical applications of liquid/solid interface chemistry including catalysis, selective adsorption of molecules, chromatographic separations, and environmental remediation, generally employ porous support materials for immobilizing ligands or reactive sites. The high surface area of porous particulate materials provides large capacities for adsorption, reaction, or catalysis. While flat surfaces are often used as models for these materials for spectroscopic investigation, the surface chemistry of porous materials cannot be totally understood by study of flat surfaces. The challenge for spectroscopic investigation of porous materials is that most (>99%) of their surface area resides within the porous structure; thus relevant methods to investigate these materials must be capable of interrogating chemistry inside the porous structure. To address this measurement challenge, we employ confocal Raman microscopy to determine the chemical composition, interface structure, and surface interactions of molecules within individual porous particles. The pore structure of these particles is much smaller than the wavelength of excitation or scattered radiation and allows diffraction-limited imaging within the particle interior. The high specific surface area of these particles (>100 m²/g) allows sub-monolayer coverages of molecules to be detected in Raman scattering without any enhancement. Confocal Raman microscopy is capable of unraveling retention mechanisms involved in chromatographic separations. It is also being developed for single-particle, solid-phase extraction to enable rapid, ultra-trace-level Raman scattering detection in sub-fL volumes. The methodology is being applied to measurements of octanol-water partitioning within silica particles whose pores filled with octanol. Finally, the method can be used to determine solute interactions with hybrid-lipid bilayers supported on n-alkyl chain ligands within individual, porous-silica particles.

Keywords: Lipids, Microspectroscopy, Modified Silica, Raman
Application Code: Materials Science
Methodology Code: Microscopy
New physical phenomena occur when the size of a structure is comparable to physical scaling lengths, e.g., the Debye length in ionic solutions, and these unique physical effects may be usefully exploited to achieve improved control over chemical manipulations, such as (bio)chemical sensing or processing. In particular, we are interested in the effects that accrue from confining reactants and products to the small volumes characteristic of 0-D and 1-D nanostructures.

Zero-dimensional architectures are also being studied in two formats: (1) arrays of nanoconfined recessed ring-disk electrodes (RRDEs) in which redox cycling can be carried out at very high efficiency; and (2) electrochemically-active zero-mode waveguides (ZMWs), in which strongly confined optical fields can be coupled to single molecule electron transfer in order to study the dynamics of single enzyme molecules and single redox-active organic chromophores. Nanoscale separation in RRDE arrays allows ultraefficient coupling of counterpoised electron transfer processes at the bottom disk and upper ring electrodes in an attoliter-volume nanopore, processes that can be exploited for improving both sensitivity and selectivity in electroanalysis. In the ZMW geometry the Au optical cladding layer is used as the working electrode in order to couple the local potential to fluorescence dynamics of molecules exhibiting large changes in emission quantum efficiency with redox state. These experiments allow the distributions of single molecule $E_{eq}$ values and other details of electron transfer reactions on a single molecule basis.

**Keywords:** Electrochemistry, Nanotechnology, Spectroelectrochemistry

**Application Code:** Nanotechnology

**Methodology Code:** Electrochemistry
The decade starting in 2000 produced one of the most exciting new developments in spectroscopy: the development of ultrafast 2D infrared spectroscopy. During that time, it went from an esoteric research tool to a commercial instrument. This talk will outline some of the principles behind the technique, the technological developments that enabled commercialization, and milestone applications.

**Keywords:** Molecular Spectroscopy, Spectroscopy, Ultra Fast Spectroscopy, Vibrational Spectroscopy

**Application Code:** General Interest

**Methodology Code:** Vibrational Spectroscopy
I will present our work in using single-molecule fluorescence microscopy to study the catalytic properties of individual metal nanoparticles at single-turnover resolution and nanometer precision. The single-molecule approach circumvents the intrinsic heterogeneity challenge among nanoparticle catalysts, so that their temporal and individual behaviors can be dissected. I will present the insights we gained into the catalytic activity and dynamics of individual metal nanoparticles, the reactivity differences of various surface sites, and the surprising spatial reactivity patterns within single facets at the nanoscale. This spatiotemporally resolved catalysis mapping also enables us to probe the communication between catalytic reactions at different locations on a single nanocatalyst, in much relation to allosteric effects in enzymes.

Keywords: Energy, Fluorescence, Microscopy
Application Code: Other
Methodology Code: Microscopy
Advances in Analytical Technology for Understanding the Central Nervous System

Evaluating and Modeling Kinetic Diversity of Brain Dopamine Systems

Dopamine (DA) is a highly significant neurotransmitter in the mammalian central nervous system, where it contributes to the control of muscle movements, learning, and mood. DA’s significance as a neurotransmitter is heightened by its role in several disorders that adversely affect human health, including Parkinson’s disease, schizophrenia, substance abuse, and depression. The DA systems are among the smallest in the brain, comprising only a few thousand neurons of the billions that exist in total, so their ability to impact in so many physiological functions is remarkable. Some evidence suggests that DA’s diversity of function is temporally encoded, i.e. that rapid DA transients and slow DA fluctuations have their own discrete functions. Thus, it is highly significant to understand the factors that control the time courses of DA function, including the kinetics of DA release, DA clearance, and DA transport in the extracellular space. Fast scan cyclic voltammetry, especially when paired with electrical stimulation of DA axons, is a proven methodology for investigating these issues. Recently, our laboratory has documented that the heterogeneity of evoked DA responses measured in the dorsal striatum, a brain region that receives the densest DA input within the CNS, is a consequence of a rich, yet previously undocumented, diversity in the kinetic properties of the DA terminal field. We find that the dorsal striatum exhibits 5 statistically distinct evoked DA responses. A single, 4-parameter kinetic model based entirely on first-order rate expressions fully captures the features of the diverse responses. Spatial mapping across the anterior-posterior, dorso-ventral, and medio-lateral aspects of the dorsal striatum is beginning to reveal the size and anatomical distributions of the diverse DA responses and their association with known patch:matrix architecture of this brain region.

Keywords: Bioanalytical, Electrochemistry, Microelectrode, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Advances in Analytical Technology for Understanding the Central Nervous System

Dopamine Release from Transplanted Neural Stem Cells in Parkinsonian Rat Striatum In Vivo

Embryonic stem cells (ESCs)-based cell therapies exhibit great potential for clinical treatment of Parkinson’s disease (PD), because they can significantly rescue PD-like behaviors. However, whether the transplanted cells themselves release dopamine in vivo remains elusive. We and others have recently induced human ESCs into primitive neural stem cells (pNSCs) that self-renew for massive/transplantable production and can efficiently differentiate into dopamine-like neurons (pNSC-DAns) in culture. Here we show that after the striatal transplantation of pNSC-DAns, (1) pNSC-DAns retained tyrosine hydroxylase expression and reduced the PD-like asymmetric rotation; (2) depolarization-evoked dopamine release and reuptake were significantly rescued in striatum in situ (brain slices) and in vivo, as determined jointly by microdialysis-based HPLC and electrochemical micro-carbon-fiber electrodes; and (3) the rescued dopamine was released directly from the grafted pNSC-DAns (not injured original cells). Thus, pNSC-DAn grafts release and reuptake dopamine in the striatum in vivo and alleviate parkinsonian symptoms in rats, providing proof-of-concept for human clinical translation.

References:

Keywords: Neurochemistry
Application Code: Neurochemistry
Methodology Code: Physical Measurements
Advances in Analytical Technology for Understanding the Central Nervous System

Optogenetic Control of Neurochemistry During Behavior

Over the past 5 years, the incorporation of optogenetics into the study of brain function has resulted in a tremendous leap in this field, initiating a revolution in our understanding of the networks underlying several cognitive processes. Here, we will present recent breakthroughs in which optogenetics was applied to research in the field of drug addiction and describe the technical approach. The nucleus accumbens (NAc) is a neural substrate involved in integrating sensory and emotional information to initiate reward-directed behavior. We previously demonstrated that dopamine release surges when an animal is presented with a cue that predicts the avoidance of punishment. However, it is not known whether this release is just an epiphenomenon of the behavior or if it has a causal role. Here, we transfected the cell bodies of dopaminergic neurons in the ventral midbrain of rats that express the enzyme cre recombinase in these specific cells, with a virus carrying a light sensitive cationic conductance. Animals were also implanted with voltammetric electrodes in the nucleus accumbens for the measurement of dopamine, and with optical fibers in the ventral midbrain to allow for specific control of these cells and not others. We found that optogenetic release of dopamine was sufficient to improve the animals' behavior by 70%. This was accompanied by a significant augmentation of subsecond dopamine release and of accumbens gamma power at the presentation of the warning signal that introduces the option to avoid. This data suggests that changes in dopamine neuron activity at specific predictors of an outcome are sufficient to produce the motor sequences to obtain it.

Keywords: Electrochemistry, Electrodes, Fiber Optics, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Serotonin is thought to be involved in the regulation of emotion-related behavior and the etiology and treatment of mood and anxiety disorders. However, direct evidence for the encoding of emotionally salient information by serotonin neurotransmission has not been forthcoming largely due to the inability to investigate serotonin signaling in a highly time-resolved manner. We have developed paradigms for measuring changes in extracellular serotonin on the minute-by-minute (fast microdialysis) and second-by-second (fast-scan cyclic voltammetry) time scales. These approaches enable us to record serotonin transmission in brain areas associated with affective and anxiety-related circuitry in behaving animals. We have developed highly resolved chemical, electrical, and optical stimulation procedures for investigating endogenous serotonin release. Moreover, we are investigating behaviorally evoked serotonin release in the context of aversive learning, dysphoria, and stress. Methods, advances, and future challenges will be discussed.

Keywords: Bioanalytical, HPLC, Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
All nervous systems employ many neuropeptides as chemical messengers. Using a sensitive mass spectrometry (MS)-based platform, more than 300 neuropeptides have been discovered in a well-defined crustacean nervous system, revealing that even a relatively simple neural network contains an unexpectedly-rich diversity of neuropeptides. To further explore the functional consequences of peptide multiplicity, MS imaging techniques and in vivo microdialysis sampling technique are employed to obtain spatial and temporal information of peptidergic signaling, respectively. Furthermore, novel isotopic and isobaric labeling techniques have been developed and employed to produce differential display of neuropeptidomes under different physiological conditions. Examples of neuropeptide regulation of feeding behavior and environmental stress will be highlighted. Collectively, these combined studies will help elucidate the functional roles that neuropeptides play in regulating neural plasticity.
Advances in Raman Raman Spectroscopy – The Synergism Between Instrumentation Evolution and Emerging Applications

The evolution of the instrumentation used to detect the Raman effect, from the initial instrument used by CV Raman himself, through to the current use of multichannel detectors on grating-based spectrographs with microscope sampling devices, will be reviewed, while indicating what fields of science have been explored during these time periods. In the earliest period we will indicate how Raman spectra were originally used as an aid to determine molecular structure. During the 1960’s it was successfully used to study the physics of semiconducting materials and devices. The introduction of the microscope in ~1974 as a sampling device first simplified experimental conditions, but also provided information on a scale commensurate with many questions of microstructure and with problems of manufacturing defects. Curiously, while the original concept of the microscope in the early 1970’s was focused on Raman imaging, technological limitations prevented its practical implementation. As the ability to acquire high quality map data over a region of interest improved, development of multivariate statistical means of treating the data has provided high quality Raman images that are now yielding solutions to problems.

Abstract Text

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Keywords: Characterization, Microspectroscopy, Molecular Spectroscopy, Raman
Application Code: Materials Science
Methodology Code: Vibrational Spectroscopy
Raman Investigation of Chemical Reactivity on the Nanometer Scale

With the help of surface plasmons molecular chemical reactions can be observed on the nanoscale. Plasmon assisted dimerization of p-nitrobenzenethiol (pNPT) molecules to 4,4-dimercaptoazobenzene (DMAB) has been realized by SERS and tip enhanced Raman spectroscopy (TERS). In the previous study, it was found that the plasmon assisted reaction can be controlled by the molecular concentration and the laser intensity. In principle, if the molecular concentration is very low, the distance of adsorbed pNTP molecules is too large for an intermolecular reaction, independent from laser power. In this case, SERS signals relate to single or several non-interacting molecules. Thus, SERS will provide information on the status of a reaction.

We present a strategy, to detect SM-SERS signals of the plasmon catalyzed chemical reaction of pNTP using gold nanoparticle dimers. When about 10 molecules are absorbed on a single dimer surface the average distance between two molecules is ~32 nm. SERS of pNTP on such a surface can be obtained at such a concentrations. Furthermore our experiments demonstrate an interesting behavior of pNTP molecules at such low concentrations upon radiation. Instead of the dimerization a different reaction can be observed, that will be discussed in detail.
Current medical imaging modalities rely on differences in tissue properties rather than on chemical-content changes deep inside the body. Consequently, a biopsy is routinely needed for ultimate diagnosis of a malignancy. Thus, we need noninvasive imaging technologies that can be used in vivo and can determine in real time the chemical content of deep-tissue. Vibrational spectroscopy has been a powerful tool for quantitative analysis of molecules in gas phase or solutions and has been deployed for label-free analysis of biological tissues. Yet, applying vibrational spectroscopy to non-invasive in vivo imaging is difficult due to limitations in imaging speed and penetration depth. In this presentation, I will present our persistent efforts in transforming molecular spectroscopy from an in vitro analytical tool to a set of in vivo label-free imaging platforms that have been enabling groundbreaking discoveries and paradigm-shifting diagnosis strategies. This transformation is achieved through development of coherent Raman scattering microscopy for real-time spectroscopic imaging of living cells and development of vibration-based photoacoustic endoscopy/tomography for bond-selective imaging of deep tissues. Potentially transformative applications to cancer metabolism, cardiovascular disease and neuroscience will be presented.
Advances in Raman Probing Low Frequency Vibrational Excitations and Their Effect on Electron and Proton Transport in Proteins

Electron and proton transport in proteins has been studied using impulsive stimulated Raman vibrational coherence spectroscopy and ultrafast kinetics. Cytochrome c (cyt c) and green fluorescent protein (GFP) are employed as model systems. “Ruffling” distortions of the heme in cyt c alter the electron transport rates by two orders of magnitude. Because this mode can also mix with other delocalized low-frequency modes of the protein, or with binding partners, it offers a potential control mechanism for electron transfer. The ruffling distortion is relaxed upon cyt c binding to the mitochondrial membrane, which suggests that et rates are enhanced in the membrane bound state. In another study, involving proton transfer kinetics, we use Duschinsky mixing of the low-frequency donor-acceptor mode with the high frequency O-H oscillator to explain the kinetic data. This quantum mixing delocalizes the tunneling wavefunction and offers shorter tunnel path-lengths, which control the underlying transport rates.

Keywords: Laser, Raman, Spectroscopy
Application Code: Biomedical
Methodology Code: Vibrational Spectroscopy
Raman spectroscopy is a powerful technique that can be used to obtain detailed chemical information about a system without the need for chemical markers. It has been widely used for a variety of applications such as cancer diagnosis and material characterization. However, Raman scattering is a highly inefficient process, where only one in $10^{11}$ scattered photons carry the needed information. Several methods have been developed to enhance this inherently weak effect, including surface enhanced Raman scattering and coherent anti-Stokes Raman scattering. These techniques suffer from drawbacks limiting their commercial use, such as the need for spatial localization of target molecules to a ‘hot spot’, or the need for complex laser systems. Here, we present a simple instrument to enhance spontaneous Raman scattering using elastic light scattering [1]. Elastic scattering is used to substantially increase the interaction volume. Provided that the scattering medium exhibits very low absorption in the spectral range of interest, a large enhancement factor can be attained in a simple and inexpensive setting. In our experiments, we demonstrate an enhancement of $10^7$ in Raman signal intensity. The proposed novel device is equally applicable for analyzing solids, liquids, and gases. Real-life applications like monitoring human waste in a pool [2] will be discussed.

References:

Keywords: Biosensors, Environmental/Water, Fluorescence, Raman
Application Code: Environmental
Methodology Code: Vibrational Spectroscopy
A short overview on general aspects of ultrafast electromigrative separations will be given followed by most recent developments of our group concerning ultrafast capillary electrophoresis coupled to mass spectrometry. The hyphenation of capillary electrophoresis (CE) with mass spectrometry (MS) is an attractive tool of instrumental analysis. However, the combination of conventional CE systems with MS is usually associated with the implementation of rather long capillaries (longer than or equal to 60 cm). Consequently, the window of migration times is typically in the range of 5-10 min or even longer. In order to speed up CE-MS separations much shorter capillaries and rather high separation voltages should be applied. A novel instrumental approach for CE-MS measurements in the time scale of seconds is presented. In addition, very small samples in the nanolitre range can be studied. Samples are handled by means of a microprocessor controlled injection system with an integrated capillary. The injection capillary is moved out of the injection cell for taking up samples from a microenvironment, and after repositioning it is facing the fixed inlet of a short separation capillary (15 cm in length), then a small sample plug is injected onto the inlet of the separation capillary. In this way separations of catecholamines could be carried out in less than 12 s. A very recent development from our laboratory is the concept of two-dimensional separations of ionic species by combining ion chromatography – fast capillary electrophoresis – mass spectrometry. This novel approach of ion analysis will also briefly be discussed.

Keywords: Capillary Electrophoresis, Electrospray, Mass Spectrometry, Small Samples
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Microchip electrophoresis is a powerful tool for the analysis of biological samples. In particular, the ability to perform fast efficient separations makes it possible to monitor several compounds simultaneously with high temporal resolution. The small dimensions of the channels in the chip are compatible with the analysis of microdialysis samples and single cells. In this presentation, two applications of microchip electrophoresis (ME) for biochemical investigations will be presented. The first application involves the development of ME-based methods for the detection of reactive nitrogen and oxygen species (RNOS) in macrophages and immune cells. These are short-lived species that have been implicated in cardiovascular disease and neurodegenerative disorders, including Alzheimer’s and Parkinson’s diseases. The second involves the combination of microdialysis with microchip electrophoresis for near real-time continuous monitoring of amino acids, catecholamines, and nitric oxide metabolites in awake, freely roaming animals. The goal of this second application is to miniaturize the entire system for simultaneous monitoring of neurochemistry and behavior with on-animal analysis and telemetry control.

Keywords: Bioanalytical, Capillary Electrophoresis, Electrochemistry, Electrophoresis
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
**Session Title**
IAEAC - Ultrafast Electromigrative Separations: Capillary versus Chip Format

**Abstract Title**
Fast Separations in Short Capillaries and Lab-On-Chip Devices With Contactless Conductivity Detection

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**Abstract Text**
In contrast to UV-detection capacitively coupled contactless conductivity detection (C4D) is readily possible in narrow channels with diameters down to 10 µm. C4D is therefore suitable for lab-on-chip devices as well as for conventional capillary electrophoresis and has frequently been employed for both. Examples for the two platforms drawn from work in our group will be presented. Fundamental aspects of C4D and how these relate to the implementation on the two electrophoresis platforms will be discussed.

In capillary electrophoresis, regardless of the arrangement employed, the speed of analysis cannot be optimized without consideration of other performance parameters, in particular separation efficiency and limits of detection. This interrelationship is not always considered, and often makes a comparison between different reported results difficult.

Other important aspects to consider are the practical handling of the two platforms with regard to flushing with electrolyte and sample injection. Manual use is cumbersome with both platforms, and for automation an external manifold is required in either case.

**Keywords:** Capillary Electrophoresis, Detector, Lab-on-a-Chip/Microfluidics, Portable Instruments

**Application Code:** High-Throughput Chemical Analysis

**Methodology Code:** Capillary Electrophoresis
Ultrafast capillary electrophoresis (UFCE) is defined as a mode of capillary electrophoresis where the field strength is $1000\, \text{V/cm}$. To maintain this field strength while using the conventional CE power supplies with a maximum of $30\, \text{kV}$, the maximum length of the capillary for the UFCE apparatus is limited to $30\, \text{cm}$. To achieve this high field strength while avoiding excessive Joule heating, narrow capillaries ($5\, \text{mm}$) are utilized. There are three main issues with these narrow capillaries: 1) They are difficult to interface to mass spectrometers without any loss of resolution or sensitivity. Our porous tip interface in conjunction with capillary format has eliminated this difficulty. 2) There is a limited sample volume that can be injected into these short, narrow capillaries, where the total volume of the capillaries is in the range of a few nanoliter or less. To increase the loading capacity of this capillary, the porous tip of the capillary is adjustable. This allows the porous tip to be pulled inside the sheath liquid for sample stacking during injection, or sample focusing in isoelectric focusing (IEF), or, alternatively, pushed outside of the sheath liquid for electrospray ionization (ESI). The adjustable interface preserves electrical conductivity during electrokinetic injection with reverse electro-osmotic flow. Therefore, pre-concentration of analytes at the capillary inlet is achieved without the need to disassemble the interface, improving the throughput. 3) There is a lack of quality control in the production of the narrow capillaries in terms of the uniformity of the inner diameter. Additionally, small particles entering the capillary or precipitation of analyte during the concentration result in frequently clogged capillaries. This issue is currently under study.
The injection method selected for an electrophoretic separation can have a dramatic impact on the resolution, throughput, sensitivity and quantitation of the analysis. We are developing new techniques that overcome many of the limitations of conventional hydrodynamic and electrokinetic injection approaches. For example, a newly developed microchip sample injector features a pneumatic microvalve that separates a sample introduction channel from a separation channel. The sample introduction channel is pressurized such that when the microvalve is briefly opened, a variable-volume sample plug is introduced onto the separation column. The microvalve is computer controlled and enables sample volumes from picoliters to nanoliters to be reproducibly injected. No quantitative bias is observed and subsequent sample plugs can be injected with minimal latency, opening the door to multiplexing approaches based on, e.g., the Hadamard transform for improved signal to noise. The microchip injector can be incorporated within monolithic microfluidic devices or seamlessly interfaced with capillary separation columns. Detection has utilized both fluorescence and electrospray ionization mass spectrometry. We have also found that samples can be preconcentrated at the closed microvalve by applying a potential across it. A 70-fold enrichment of a model analyte was achieved in just 8 s. The concentrated analyte was then injected as a 1 nL plug for CE. These advances should enhance the performance of both microchip and capillary-based CE and are also expected to lead to greatly improved multidimensional separations (e.g., liquid chromatography-CE).

Keywords: Capillary Electrophoresis, Electrospray, Lab-on-a-Chip/Microfluidics, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
By conjugating gold or silver nanoparticles to different locations of living phase synchronized cells, (e.g., cancer cells), one can use SERS to study the time sequence of the different processes occurring in a live single cell, e.g., drug efficacy,[sup]1[/sup] drug delivery,[sup]2[/sup] or the mechanism(s) of cell death process itself.


Keywords: Biospectroscopy, Medical, Nanotechnology, Surface Enhanced Raman
Application Code: Nanotechnology
Methodology Code: Molecular Spectroscopy
Multifunctional nano-carriers enabled a major route for the achievement of theranostics, i.e., simultaneous therapy and diagnostics, where the later can be broadly defined as covering structural (physical) and functional (chemical) imaging, surgery assisting delineation, as well as monitoring of therapy. The mode of therapy can be chemical (e.g. drugs), physical (e.g. radiation), or a combination (e.g. photochemical). Drug targeting, to specific organs, cells, organelles, or cell biomolecules, has been the dream of the biomedical community for a century; it has been transformed into reality only recently, largely with the use of “nano-drugs”. A nanoparticle based platform can include drugs, drug sensitizers, imaging contrast agents, targeting moieties, “cloaking agents” and more. This recent development of nanoplatforms has made possible such combinations of therapeutic functions, as well as better designed biodegradation and bioelimination. Application examples include: (1) brain cancer optical and photoacoustic imaging, surgical delineation, chemotherapy and photodynamic therapy (PDT), (2) ovarian cancer chemotherapy, (3) optimization of cancer cell photothermal therapy via nuclear delivery of surface modified Au nanoparticles, (4) targeted PDT of arrhythmia causing cardiac cells in-vivo, (5) chemical imaging of hypoxia and acidity by photoacoustic imaging, (6) Cell Magneto-Rotation (CMR) for evaluating the metastatic potential of individual breast cancer cells.
The great promise of single-molecule/assembly measurements is to understand how critical variations in structure, conformation, and environment relate to and control function.\(^1\) New approaches to imaging and analysis are keys to elucidating these associations. I will discuss current and upcoming advances and will pose the challenges that lie ahead in creating, developing, and applying new tools for biology and medicine. These advances include fusing spectroscopic imaging modalities and freeing up bandwidth in measurements to record simultaneous data streams and to expand our dynamic range. Recent advances in sparsity and compressive sensing can be applied both to new analysis methods and to directing measurements so as to assemble and to converge structural and functional information. Early examples will be discussed.

A full understanding of the molecular basis of diseases depends on the development of molecular probes able to recognize disease targets of interest. Until very recently, such tools have been absent from the clinical practice of medicine. The newest molecular probe, and one that holds most promise, is a new class of designer nucleic acids, termed aptamers, which are single-stranded DNA/RNA able to recognize specific targets, such as single proteins and even small molecules. Recently, we applied a simple, fast and reproducible cell-based aptamer selection strategy called Cell-SELEX which uses whole, intact cells as the target for aptamer selection. This selection process then generates multiple aptamers for the specific recognition of biological cells, but without the need for prior knowledge about the signature of target cell-surface molecules. The selected aptamers have dissociation constants in the nanomolar to picomolar range. Thus far, we have selected aptamer probes for many different diseases, and used them to carry out studies at the vanguard of biomedical science, including ultrasensitive detection of tumors, molecular imaging, targeted drug delivery, and, most critically, cancer biomarker discovery. Taken together, these molecular level tools form a solid scientific platform from which to pursue advanced studies in molecular medicine. We will report our most recent progress in this exciting research area, especially the molecular elucidation of cancer biomarkers and targeted drug development.
A novel class of metal-free spherical nucleic acid nanostructures was synthesized from readily available starting components. These particles consist of 30 nm liposomal cores, composed of an FDA-approved 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) lipid monomer. The surface of the liposomes was functionalized with DNA strands modified with a tocopherol tail that intercalates into the phospholipid layer of the liposomal core via hydrophobic interactions. The spherical nucleic acid architecture not only stabilizes these constructs but also facilitates cellular internalization and gene regulation in SKOV-3 cells.
Nanocrystals in Energy Generation and Storage: Fuel Cells and Batteries

Nanostructured materials are key elements of energy generation and storage technologies. Their performance, in terms of activity and durability, is critically dependent on composition and structure, the nature of the surface, the interface and its morphology. This presentation will deal with the synthesis and in situ/operando characterization of novel materials for fuel cells and batteries employing ordered intermetallic phases and nanostructured materials. In the case of fuel cells, we will focus on the oxygen reduction reaction (ORR) using Pt/M (M = Fe, Ni, Co, Cu) alloys as well as ordered intermetallic core/shell nanostructured electrocatalysts (1-3). Variations in electrocatalytic activity will be discussed within a framework of electronic effects and lattice strain. Operando methods, with emphasis on TEM will be presented and degradation mechanisms proposed. In the case of batteries, the presentation will focus on a detailed mechanistic study of the conversion reactions of Mn\(_3\)O\(_4\) (4) as an anode material as well as in situ/operando lithiation dynamics of LiFePO\(_4\) (5). The presentation will conclude with an assessment of future directions.

Keywords: Energy, Fuels\Energy\Petrochemical
Application Code: Other
Methodology Code: Electrochemistry
Recently, we have shown that it was possible to couple a redox flow battery with two external circuits to carry out indirect electrolysis. The gist of this approach is to store “junk electricity” in a redox flow battery when its prize from the grid or from renewable sources is very low and to use this stored redox energy to carry out indirect electrolysis.

For example, with a vanadium redox flow battery, Vanadium(II) can be used to produce hydrogen whilst Vanadium(V) can be used to oxidise SO2 to generate protons and sulfuric acid. To perform these reactions in external reactors, it is important to use nanoparticle catalysts.

We shall discuss here nanoelectrocatalysis with nanoparticles supported on silica or other ceramics. Two redox half-reactions take on the nanoparticle, e.g. hydrogen evolution and V(II) oxidation, and we shall discuss both the thermodynamic and the kinetic aspects. More generally, we shall discuss the role of nanoparticles in the field of redox flow batteries and possible applications for energy storage.

**Keywords:** Electrochemistry, Energy, Fuels\Energy\Petrochemical, Nanotechnology
**Application Code:** Fuels, Energy and Petrochemical
**Methodology Code:** Electrochemistry
To better understand the fundamental properties, correlate structure-function relationships of nanoparticles (NPs) and optimize NPs activity for various applications analytical tools that enable precise characterization of individual NPs are of immense importance. The development of "electrocatalytic amplification" is one such method where single NPs are detected by measuring electrocatalytic current (or potential) due to the electrochemical processes (oxidation/reduction of the species present in solution) occurring on the surface of the NP whenever a NP collides with an inert ultramicroelectrode (UME) which otherwise cannot catalyze the reaction. Here we report the influence of ligand, and morphology of the Pt NP on the current-time (i-t) response during single Pt NP collisions with the Hg UMEs. We evaluated the catalytic activity of ~11 nm spherical Pt NPs capped with different ligands (citrate, polyvinylpyrrolidone (PVP), polyacrylate (PA) and tetradecyltrimethylammonium bromide (TTAB)). Pt NPs of different shapes (hexagons and cubes) were also studied, as well as, Pt NPs without ligands referred as "naked Pt NPs". Hexagonal Pt NPs possess (100) and (111) facets where cubic Pt NPs possess (111) planes as preferential surface facets. The integrated charge passed per spike during single NP collision for polyacrylate capped hexagonal and cubic Pt NPs concentrated over a range of (120-210) and (40-120) pC respectively. Whereas, for naked hexagonal and cubic Pt NPs the integrated charge passed per spike during single NP collision concentrated over a range of (180-400) and (60-200) pC respectively. Naked Pt NPs displayed higher catalytic activity for hydrazine oxidation compared to polyacrylate capped Pt NPs of similar size and size. Also, hexagonal Pt NPs displayed higher (two times) catalytic activity for hydrazine oxidation compared to cubic Pt NPs.

Keywords: Electrochemistry, Electrode Surfaces, Electrodes
Application Code: Nanotechnology
Methodology Code: Electrochemistry
Spatially resolved electrochemical experiments at the single nanoparticle level are essential for uncovering relationships between catalyst structure and performance. We used nanometer-sized electrodes and nanopipettes as tips in the scanning electrochemical microscope (SECM) to visualize and quantitatively measure catalytic activities of individual metal nanoparticles. New methodology was developed for detecting charged reactive intermediates and applied to mechanistic analysis of oxygen reduction reaction. A nanopipette filled with organic phase immiscible with the external aqueous solution was used to deliver neutral redox species (oxygen) to the electrocatalytic surface and detect negatively charged intermediate (superoxide). The developed approach is suitable for mechanistic studies of other electrocatalytic processes and other heterogeneous reactions involving ionic intermediates.

Keywords: Electrochemistry, Imaging, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Electrochemistry
MXenes, a recently discovered large family of two-dimensional (2D) early transition metal carbides and carbonitrides, have shown much promise in electrochemical energy storage applications, such as battery and supercapacitor electrodes. We recently reported on large volumetric capacitance and high rate capability of Ti3C2Tx – the most studied MXene to date. Spontaneous intercalation of a variety of single- and multiply charged cations, together with highly reversible electrochemical insertion of the same cations, has been well documented for Ti3C2Tx in aqueous electrolytes. Perfect capacitive behavior was observed for Ti3C2Tx MXene even at quite high charge and discharge rates, contradicting to slow intercalation of ions in a specific potential range, which is usually observed in layered materials for battery applications.

In order to understand mechanism of capacitance in MXenes we performed characterization of the mechanical deformations of MXene electrode materials at various states-of-charge with a variety of cations (Li, Na, K, Cs, Mg, Ca, Ba, and three tetra-alkylammonium cations) during cycling by electrochemical quartz-crystal admittance (EQCA, quartz-crystal microbalance with dissipation monitoring) combined with in situ electronic conductance and electrochemical impedance. Based on this work, it appears that in MXenes cationic insertion is accompanied by significant deformation of the Ti3C2Tx particles, that occurs so rapidly so as to resemble 2D ion adsorption at solid-liquid interfaces. The latter is greatly facilitated by the presence of water molecules between the MXene sheets.

**Keywords:** Electrochemistry, Energy, Voltammetry

**Application Code:** Materials Science

**Methodology Code:** Electrochemistry
Carbon nanomaterials have potentially useful electrochemical properties, including fast electron transfer kinetics and high surface area. The use of carbon nanotubes (CNTs) in fabricating large electrodes has been widely demonstrated but the reproducible production of small, sensitive CNTs-based sensors is not as well studied. Our lab has investigated several different techniques for making CNT-based microelectrodes and investigated their response using fast-scan cyclic voltammetry. We have developed vertically-aligned CNTs on metal substrates as microelectrodes with chemical vapor deposition (CVD). The alignment of CNTs and the inherently high conductivity of metal substrates determine the sensitivity of the microelectrodes for nano-molar detection of neurotransmitters. We have also used CNT yarns/fibers as microelectrodes due to their different adsorption/desorption properties for dopamine that allow them to be used with higher temporal resolution without a decrease in sensitivity. Thus, CNT-based microelectrodes are advantageous as high sensitivity and high temporal resolution electrochemical sensors for neurotransmitters. Other types of carbon nanomaterials could be advantageous for microelectrode sensors as well, such as a different type of edge-plane graphene, termed carbon nanospikes. Carbon nanospikes are formed with plasma-enhanced CVD and do not require a catalyst. Preliminary electrochemical tests reveal that they have promising electrochemical properties that depend on the metal substrate they are deposited onto. Overall, this talk will explore the development of carbon-nanomaterial based microelectrodes for neurotransmitters detection. The ease of making different types of microelectrodes will be compared, as well as the electrochemical properties and implications for their use in vivo.

Keywords: Bioanalytical, Electrode Surfaces, Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Implantable Conductive Polymer Electrodes for In Vivo Measurements

Conducting polymer electrodes have emerged as an attractive alternative to metal and carbon-based electrodes in a variety of applications. Conducting polymer electrodes are inexpensive, easy to process, and are robust. Additionally, they have electron transfer kinetics appropriate to measure the redox properties of biologically relevant molecules. The poly(3, 4-ethylenedioxythiophene), PEDOT, family of conducting polymers offers the additional benefits of high optical transparency, electrochromism, thermal stability up to 260 °C, and demonstrated biocompatibility. PEDOT is most commonly dip-coated or electropolymerized onto metal or carbon electrodes before being employed for electrochemical measurements. PEDOT-only band electrodes (i.e. those without a conducting metal under-layer) have emerged recently and seen use as an electrochemical sensor. Other applications of structured conducting polymer films include antistatic coatings, ITO-free and ITO-containing photovoltaic cells, capacitive touch screens in mobile devices, capacitors, lead-free solder, electrochemical actuators, and flexible displays in organic light emitting devices. Here we fabricate three dimensional PEDOT-based electrode arrays for in vivo monitoring of neurotransmission.

Keywords: Bioanalytical, Electrochemistry, Microelectrode, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
The brain functions through a series of complex biochemical interactions that underlie organized neuronal circuits. Disruptions in these signaling pathways can result in dysfunction. As such, measuring the dynamic biochemical signaling in the brain is expected to provide critical information for understanding and treating numerous neuropsychiatric disorders. Unfortunately, the tools for measuring dynamic biochemical signaling in functioning tissue are still very limited. To meet this critical need, our group has been creating and adapting microfabricated electrochemical sensors for a variety of applications, effectively expanding the number and type of biochemical measurements that can be made in vivo utilizing electroanalytical techniques. We utilize fast scan cyclic voltammetry, a proven in vivo technology with excellent performance for our analytes of interest. Microfabrication enables the dimensions and geometry of the sensor to be precisely defined, improving sensor-to-sensor repeatability and enabling new sensor geometries to be created and tested. Recent efforts have focused on creating sensors that enable metabolic markers and monoamine neurotransmitters to be monitored on all time scales. This presentation will describe the creation and application of these microfabricated sensors to a variety of biochemical measurements in vivo.

**Keywords:** Bioanalytical, Electrochemistry, Nanotechnology, Neurochemistry

**Application Code:** Neurochemistry

**Methodology Code:** Electrochemistry
The importance of the so-called reactive nitrogen and oxygen species (RNOS) in biology and food technology has been widely recognized. However, when these species are in excess, the steady-state maintained by physiological processes is disturbed, leading to nitro-oxidative reactions in food and in living organisms. Analytical methods enabling the assessment of the antioxidant activity of a biological sample is therefore largely sought after. Among these RNOS, peroxynitrite (ONOO⁻) is a well-known inflammatory mediator making the development of peroxynitrite specific sensors of urgent demand. Though several strategies have been developed for peroxynitrite detection, electrochemical quantification has come into the forefront of interest when it comes to real-time, label-free and direct measurements of these reactive species.

This presentation investigates the electrocatalytic activity of metallophthalocyanine modified rGO hydrides towards peroxynitrite detection. Besides careful functionalization of rGO, strategies for the optimization of the interface design will be discussed.

References:
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Keywords: Bioanalytical, Biosensors, Chemically Modified Electrodes, Electrochemistry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Norepinephrine (NE) is released by sympathetic nerves supplying small mesenteric arteries (MA) and veins (MV). We used continuous amperometry with a diamond microelectrode to study temperature sensitive mechanisms of NE release and clearance at the adventitial surface of isolated rat MA and MV in vitro. In this measurement, NE release is evoked by electrical stimulation and a fraction of the released NE diffuses from the release site and is measured as an oxidation current. NE release in MA and MV was blocked by the calcium channel blocker, CdCl2 and by the sodium channel blocker, tetrodotoxin. NE current at MV was greater than at MA, and NE current at MV exhibited greater temperature sensitivity than at MA. At 37 °C, the rate of NE release was significantly greater at MV than that at MA but NE clearance at MV was slower. At 28 °C, there were no artery-vein differences in NE release or but clearance was slower in MV. The 2-adrenergic receptor antagonist, idazoxan (1µM), increased NE peak current and the rate of rise at MA at 28 and 37 °C but not at MV at either temperature. The Gi/Go protein blocker, pertussis toxin (3 µg/ml), increased NE current in MA but not MV at 28 and 37 °C. The NE uptake inhibitor, cocaine (10µM), increased peak NE currents and their rate of rise in MA at all temperatures. NE overflow in MV was unaffected by cocaine. The decay time of NE current was prolonged at MA but not MV at both temperatures. Guanethidine inhibited NE release through NE transporter in both MA and MV. The blockade of both NET and presynaptic 2 receptor at the same time in MA increased the current amplitude, rise time and decay time at both 28 and 37 °C, but current rise slope was only increased at 28 °C. The difference of temperature dependent NE current sensitivity between MA and MV disappeared after cocaine treatment with and without idazoxan. We conclude that the NE release and clearance mechanisms are different in sympathetic nerves associated with MA and MV.

Keywords: Electrochemistry, Electrodes, Materials Science, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
The QbD approach including application of design of experiments (DOE) concepts for the development of analytical methods for product characterization, routine product release, and stability studies have recently gained momentum within the pharmaceutical and biopharmaceutical industry. The paper will provide a review of QbD based method development performed for injectable drug products. QbD concepts from ICH Q8, Q9, and Q10 can be applied to analytical method development for pharmaceutical drug products. A method development cycle can be significantly reduced yielding savings in time and money. Potentially, the QbD approach may be applied to non-chromatographic methods. ICH guidance would be useful in streamlining the QbD process and also to alleviate concerns over “How much extra work is needed” to implement QbD.

Keywords: Analysis, Chromatography, HPLC, Optimization
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Recent Initiatives by FDA and Compendia: How do They Impact Analytical Development for Pharmaceuti
IQ Consortium Initiatives with Respect to AQbD/Analytical Method Lifecycle Management

There has been a great deal of interest in Analytical Quality by Design (AQbD) and Analytical Method Lifecycle Management in the past few years, including the recent publication of the USP and FDA draft guidances on Analytical Lifecycle Management and Analytical Method Validation, respectively. Additionally, a collaborative group of pharmaceutical representatives was formed from the IQ Consortium to discuss these topics. One outcome of the discussions was the development of a risk-based strategy for assessing analytical method comparability and the management of analytical methods changes. In this presentation, we will highlight the risk-based strategy discussed surrounding analytical method comparability and will share additional discussion topics and activities by the IQ analytical working group surrounding AQbD and Analytical Method Lifecycle Management.

Keywords: Drugs, Method Development, Pharmaceutical, Validation
Application Code: Pharmaceutical
Methodology Code: Separation Sciences
The lifecycle concept described in ICH Q8, 9&10 is adaptable to analytical procedures. The purpose of applying lifecycle principles to analytical procedures is to holistically align analytical procedure variability with the requirements of the product to be tested, and improve the reliability of the procedure by understanding, reducing and controlling sources of variability. Recently, USP initiates discussion towards adoption of these concepts in its vision of validation, verification and transfer of analytical procedures (chapters <1224>, <1225> and <1226>). During this presentation, an update of the current state of the discussions will be presented.

Keywords: Quality Control, Validation
Application Code: Validation
Methodology Code: Data Analysis and Manipulation
Recent Initiatives by FDA and Compendia: How do They Impact Analytical Development for Pharmaceuticals

Why the Tolerance Intervals Fill the Gap Between Method Validation and QbD Guidances: The USP Trend

Regarding the validation of analytical procedures, there are actually two – almost contradictory - trends existing: the ICH Q8 Quality by design thinking and the ICH Q2 guidance. Many dimensions do oppose two set of thinking: The QbD has a goal-oriented and risk-based thinking to assure quality of each future outcome while ICH-Q2 is based on the state of the art thinking and evaluation of average performance of an analytical procedure. On one hand the quality of each measure matter, on the other hand only the average estimated performance matter. On one hand the way to develop and to continuously improve is central to the concept of quality while for ICH Q2, development is not part of the picture and improvement is not envisaged either: once a method is validated it is forever.

The USP new guidance aim at filling the gap by giving to the analytical procedure a lifecycle approach and introduce the concept of risk in the decision making process. Central to this idea is the use of the Tolerance interval to make a decision about the future quality of the results. The Tolerance Interval is in reality a prediction interval and it gives an idea about the probability of each measure to fall within the confidence interval. The allows to make the connection between the estimated performance of an analytical procedure (bias, precision) and the future chance or probability that each result in the future will be within the specification limits. In addition, as it will be shown, the tolerance interval has an impact on the lifecycle approach. Indeed the same tolerance interval can also be used to define control limits for future continued improvement of the analytical method. Finally, connections with the concept of uncertainty and risk-based management will be established. Globally, the presentation will attempt to show how UPS current trends in documents such as <1210> is not only providing new insights but allows to fill the gaps between the various concepts in place.

**Keywords:** Chemometrics
**Application Code:** Pharmaceutical
**Methodology Code:** Data Analysis and Manipulation
Celebrating Diversity through Diverse Analytical Chemistry

This talk will serve as an introduction to a session celebrating diversity in analytical chemistry by focusing on the diversity – or increased need for - in the chemical sciences. Features of the current landscape, experiential knowledge, and evidence-based findings will be highlighted, with an aim to raise the consciousness of the chemical community to the complex challenges faced by underrepresented groups in the STEM disciplines. The purpose of this talk, and the session as a whole, is to better our current state of understanding of strategies that support and enhance the recruitment, retention, and advancement of underrepresented groups in STEM.

Keywords: Education
Application Code: Other
Methodology Code: Education/Teaching
Celebrating Diversity through Diverse Analytical Chemistry

Analytical Chemistry Employing Ionic Liquids and GUMBOS

My research group has been exploring the analytical applications of room-temperature ionic liquids (RTILs) for several years. Recently, we have extended the range of these materials to include analytical applications of similar solid phase materials, i.e. organic salts with melting points of solid ionic liquids (25 °C to 100 °C) and other organic salts up to melting points of 250 °C. Since most of these materials cannot be classified as ionic liquids by the current definition (melting points below 100 °C), we contrast these new materials from RTILs by creating a new acronym, GUMBOS (Group of Uniform Materials Based on Organic Salts). These GUMBOS have the tunable properties frequently associated with RTILs, including tunable solubility, melting point, viscosity, thermal stability, and functionality. However, these materials allow the production of solid phase materials which have a wider range of applications in measurement science, thus extending the task specific properties of ILs to the solid phase. In this talk, I will highlight recent applications of GUMBOS which we have explored in the area of measurement science, including GUMBOS as sensors, imaging agents, stimuli-responsive materials, and for production of nanoGUMBOS. In regard to the latter, we believe that our methodology represents an extremely useful approach to production of nanomaterials since our materials are designed and assembled for task specific uses, rather than adapted for such use as is done for many nanomaterials. Selected examples of such applications will be highlighted in this talk. Particular emphasis will be placed on a novel QCM sensor for measurement of volatile organic compounds with simultaneous molecular weight determination and development of sensor arrays for measurement of analytes in gaseous and aqueous media.

Keywords: Bioanalytical, Biosensors, Volatile Organic Compounds, Wet Chemical Methods

Application Code: Bioanalytical
Methodology Code: Chemical Methods
Celebrating Diversity through Diverse Analytical Chemistry

Kinetic Intermediates of Peptides and Proteins Using HDX-TIMS-MS

Recent innovations in speed, accuracy and sensitivity have established mass spectrometry (MS) based methods as a key technology for the analysis of kinetic intermediates and folding mechanism of protein complexes. When complemented with theoretical calculations, Ion Mobility Spectrometry – Mass Spectrometry provides a powerful tool for the identification of structural motifs. We have recently introduced Trapped Ion Mobility Spectrometry coupled to Mass Spectrometry (TIMS-MS) as a high-throughput technique for the study of solution states of biomolecules (e.g., peptides, proteins, DNA and their complexes), as well as the kinetic intermediates involved during their folding as a function of the molecular environment (e.g., pH, organic and salt content). While this description holds true for most contemporary IMS analyzers, the higher resolving power (e.g., R = 150-250, 3x larger than traditional IMS systems) and the unique ability to hold and interrogate molecular ions for kinetic studies (e.g., millisecond-second time scale) provides TIMS-MS with unique capabilities (e.g., recently combined with hydrogen-deuterium exchange, HDX-TIMS-MS and selective dopants in the TIMS cell). That is, HDX-TIMS-MS has a significant advantage in the flexibility to interrogate, at the single molecule level, the molecular interactions that define the conformational space.

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Keywords: Instrumentation, Mass Spectrometry, Method Development, Protein
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Cells carefully control their architecture by restricting the localization of protein components. Both mammalian and bacterial cells are able to do this by enclosing proteins within, or associating proteins with, lipid bilayers. However, membrane proteins are notoriously difficult to analyze due to their hydrophobicity and/or denaturant-resistant properties. Organelle proteomics, which couples biological isolation methods with mass spectrometry techniques, has been successfully utilized to more fully elucidate the composition of distinct subcellular regions. Yet, additional novel protocols are necessary to characterize recalcitrant structures and dynamic internal associations. This presentation will highlight the diversity of innovative approaches developed and used by our laboratory to effectively enrich, identify, and characterize membrane-associated proteins from (1) the gram-negative bacterium \textit{Caulobacter crescentus}, (2) rodent mitochondria-ER contact sites known as mitochondria-associated ER membranes (MAM), and (3) rodent liver microsomes.
Currently, there are approximately 50 proteins encoded in the human genome that contain the catalytic SET (Suppressor of variegation, Enhancer of zeste, Trithorax) domain, characteristic of nearly all KMTs. Roughly about a fourth of these KMTs have been shown to methylate histone proteins substrates to regulate gene expression. However, despite widespread efforts, only a small number of non-histone protein substrates for any KMT are defined. The significance of KMT activity in both normal physiology and disease is emerging as highly significant, as association of over 25 KMTs with a multitude of different human cancers have been reported. Therefore, an unambiguous determination of KMT activity, target sites and induced cellular responses or phenotypes would be highly beneficial to the chromatin, cancer and clinical biology communities, as this information is severely limited. We have recently developed novel quantitative affinity mass spectrometry (MS) based proteomics approaches to characterize in vivo protein lysine methyltransferase (KMT) activity in human cancers on both histone and non-histone proteins. Discussed will be the methods that we have developed to quantify histone modification patterns and also different methods to identify hundreds of non-histone methylated proteins in human cells, the most comprehensive large scale global analysis of protein lysine methylation (e.g. the “methylome”) to date, including KMT specific methylomes.

Keywords: Mass Spectrometry, Proteomics
Application Code: Genomics, Proteomics and Other ‘Omics
Methodology Code: Mass Spectrometry
As a leading cause of death in developing countries and a persistent problem elsewhere, pathogenic organisms are as ubiquitous as they are dangerous. Significant worldwide resources are directed toward their detection and eradication, leading to a broad coalition of government agencies, healthcare providers, academic researchers, and food manufacturers dedicated to providing the best-available prevention strategies for mitigating exposure risk. At the forefront of this effort is the field of pathogen detection. Our work directly addresses the current lack of simple, rapid, sensitive, and selective pathogen detection methods needed for frontline intervention in the most at-risk populations. To begin, we focused on the common food- and water-borne contaminant, E. coli. Using a resonance energy transfer system incorporating a bioluminescent protein and quantum dots, we demonstrated that adjacent hybridization of sequence-specific, labeled probes could detect E. coli 16s rRNA at concentrations as low as 2.1 nM in only 5 minutes. Continuing, we developed a novel tuberculosis (TB) biosensor in both microtiter plate and paper-based microfluidic platforms for the detection of two different TB DNA biomarkers. The dual-platform design afforded either quantitative (microtiter plate, 1.0-20.0 nM) or qualitative (paper microfluidic) detection. Finally, we extended the paper-based platform concept to Epstein-Barr virus (EBV) detection using a target-bridged capture scheme in which EBER-1 RNA from EBV linked a tethered probe to a fluorescent reporter probe for a low nanomolar detection limit. While divergent in design and target, these assays achieve the aims of current pathogen detection research while providing cost-effective options for deployment in resource-poor environments.

Keywords: Bioanalytical, Biological Samples, Biomedical, Biotechnology
Application Code: Biomedical
Methodology Code: Sensors
Celebrating Diversity through Diverse Analytical Chemistry

A Voltammetric Characterization of Serotonin’s Roles in Depression and Neurodegenerative Diseases Associated Depression

Serotonin is a neuromodulator that is most notoriously involved in depression. Depression affects up to 15% of the US population and is particularly prevalent (up to 50%) in Parkinson’s Disease (PD) patients. Fast-scan cyclic voltammetry (FSCV) tracks serotonin concentrations in real-time in living mammalian brains. In this work, we direct a voltammetric analysis towards serotonin in mouse models of PD in the substantia nigra (SN). We assess the effects of induced PD and PD therapy (L-Dopa) on serotonin levels and find that serotonin neurotransmission is significantly altered in PD, correlating with time after disease onset. Furthermore, we find that L-Dopa therapy significantly exacerbates this alteration in serotonin neurotransmission. Via mathematical modeling, we propose mechanisms for PD related depression and discuss the relevance of antidepressants to this pathophysiology.

Abstract Text

Bioanalytical, Microelectrode, Neurochemistry, Voltammetry

Application Code: Neurochemistry

Methodology Code: Electrochemistry
Celebrating Diversity through Diverse Analytical Chemistry

Innovation and Research: Converting Science to Meaningful Solutions

The Environmental Health division of PerkinElmer offers a wide range of technology platforms including IR and UV spectroscopy, LC and GC, mass spectrometry (MS), elemental analysis platforms such as AA, ICP-OES, ICP-MS, and a wide variety of software and service solutions. This broad portfolio serves a wide variety of applications in many different markets. Understanding and anticipating customer requirements, translating them to technology needs, and executing Research and Development (R&D) projects to invent new technologies and solutions that meet or exceed customer demands is an essential part of our business. In this presentation, you will get an overview of PerkinElmer’s solutions for different applications and will understand the role that innovation and early-phase research plays in coming up with truly innovative products in rapidly-changing markets.

Keywords: Elemental Analysis, Gas Chromatography, Spectrometer, Spectroscopy
Application Code: Environmental
Methodology Code: Other
Most often sensing with nanopores implies monitoring changes in the voltage driven ion flow across a single nanopore that separates two electrolyte chambers as target species are passing through or residing within the nanopore sensing zone. This versatile technique for studying and quantifying nanoparticles of synthetic or biological origin approaches the limits of practical applicability when used for the detection of small ions and polyelectrolytes. Instead of measuring extremely small current pulses here we present new sensing schemes based on using chemically-modified nanopores and potentiometric transduction of the selective recognition reactions within the nanopore. Beside potentiometric detection of ions and polyelectrolytes we are proposing alternative signal amplification schemes for quantification of polyelectrolytes using nanopores modified with selective artificial receptors.

Keywords: Biosensors, Electrochemistry, Lab-on-a-Chip/Microfluidics, Nanotechnology

Application Code: Bioanalytical

Methodology Code: Electrochemistry
Ion-selective electrodes have been well established in a variety of analytical applications and a range of selective materials have been developed that allow these systems to exhibit the desired selectivity and detection limits for a range of target samples. Direct potentiometry, however, is prone to potential drifts and does not provide diagnostic information on the type of analyte that is detected. It is also not a technique useful for multianalyte detection.

This talk aims to describe concepts that may result in calibration-free multianalyte detection using ion-selective membranes. The goal is achievable with thin layer voltammetry at ion-selective membranes, where the sample composition is exhaustively altered in the course of the measurement. Diagnostics on the identification of the detected ions is possible by the resulting peak potentials. Early examples of this principle will be presented.

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**Keywords:** Bioanalytical, Electrochemistry, Ion Selective Electrodes

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
In this work, two ion-transfer reactions at polymeric membranes are diagnosed voltammetrically to develop ultrasensitive ion-selective electrodes with subnanomolar and picomolar detection limits. On one hand, we demonstrate that perfluoroalkyl sulfonates and carboxylates are readily extracted into an ionophore-free membrane owing to the high lipophilicity of these emerging environmental contaminants. The extraction process, however, is limited by the moderate solubility of the fluorophilic ions in the lipophilic membrane, which explains why no response was obtained in ion-selective potentiometry. On the other hand, polypeptide protamine is only adsorbed at ionophore-based membranes in contrast to the traditional belief that the biomedically important polycation is extracted into the membranes for potentiometric and optical sensing. The voltammetric approach not only leads to the greater understanding of the membrane behaviors of the important analyte ions but also enables their ultrasensitive detection based on stripping voltammetry.

Keywords: Electrochemistry, Electrodes, Environmental/Biological Samples, Sensors
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Ionophore-Based Chemical Sensors I

Ion-Selective Optodes Based on Reversible Meta-Stable Photoacids

Photodynamic molecules have far-reaching implications in medicine, forensics and environmental sciences. Advances in photochemistry and synthetic methodology promise molecules with tailored properties which can virtually cater to almost any application. Our interest lies in using reversible meta-stable photoacids in dynamic ion-selective optodes which can be used for the wireless detection of essential and toxic metal ions. The use of photoacids enables the sensor to be switched ON/OFF on demand while providing for spatial and temporal control. Our studies have open three directions of exploration. The first is the modulation of equilibrium response of the sensor by appropriate functionalization of the photoacids with groups having various electron demands. This enables us to tune equilibrium responses tailored to the time frame of the application. The second direction aims to solve leaching issues by covalently linking the photoacid to the sensor matrix. This approach also enables us to tailor the lipophilicity of the photoacid in the matrix. The third direction uses non-ionic photoacids which promises universal compatibility with all types of plasticizers, enhanced lipophilicity and may ultimately lead to pH independent sensing. The sensors we have designed so far are highly selective, have low limits of detection and are activated by visible light. Future work is aimed at miniaturization of these sensors as well as designing photoacid optodes which can be switched on in the near IR region.

Keywords: Bioanalytical, Quantitative, Spectroscopy, UV-VIS Absorbance/Luminescence

Application Code: Bioanalytical

Methodology Code: Sensors
The presentation discusses the hydrophobic properties of different types of ion-to-electron transducer materials. The main focus is on polyazulene (PAz) [1] and polyaniline (PANI) [2,3] based materials, and composites consisting of graphene derivatives and electrically conducting polymers (ECP) [4-6]. In the latter, the ECP takes care of the ion-to-electron transduction while the graphene derivatives improve the pseudocapacitance, hydrophobicity and mechanical stability of the transducer. A few case studies with preliminary results will be briefly presented where PAz and the composite materials have been applied as transducers in polymeric solid-contact ion-selective electrodes. Finally, different strategies will be shortly discussed for reducing and mapping the water uptake of polymeric ion-selective membranes.


Keywords: Ion Selective Electrodes, Materials Science, Potentiometry, Sensors
Application Code: Bioanalytical
Methodology Code: Sensors
Micro- and nanostructures are reaching considerable attention in context of sensing applications. Either linear dependence of signal on logarithm of concentration within the broad range or sigmoidal shape dependence within rather limited (fixed) range are reported [e.g.1,2,3]. The aim of this work was to investigate the possibility of modify sensitivity and response range of polymeric micro- and nanospheres. To achieve this goal in principle different approaches are available. Optimizing material used to prepare micro- or nanospheres is one of them. For “classical” microspheres change of the polyacrylate material (e.g. from poly(n-butyl acrylate) to poly(lauryl acrylate) can result in substantial differences in ion-permeability and ultimately in optical responses of microspheres.

The alternative is to use other materials like e.g. alternative polymers, to prepare nanospheres, with lipophilic core and ion-exchanging surface. This approach has resulted in fluorimetric sensors of broad responses range. The choice of polymer used of prepare nanospheres is shown to affect the response range and sensitivity of prepared sensors. Potassium or calcium ions sensors prepared were characterized with stable response and broad linear range of emission change accompanying change of logarithm of analyte sample concentration.


Keywords: Fluorescence, Materials Science, Sensors, UV-VIS Absorbance/Luminescence
Application Code: Biomedical
Methodology Code: Fluorescence/Luminescence
The surface chemistry of nanoscaled plasticized poly(vinyl chloride) and unplasticized poly(methyl methacrylate)/poly(decyl methacrylate) membranes doped with redox molecules such as ferrocene and new hydrophobic cobalt organometallic compounds has been studied using cyclic voltammetry (CV), synchrotron radiation-X-ray photoelectron spectroscopy (SR-XPS) and near edge X-ray absorption fine structure (NEXAFS). This paper will present interesting findings on mass transportation effects to and from within the nanoscaled membrane films.
A calibration-free ion-selective electrode measurement requires that only the phase boundary potential at the sample/sensing membrane interface is variable (depending on the activity of the target ion in the sample) while all the other phase boundary potentials in the electrochemical cell consisting of the indicator electrode, the reference electrode, and the sample are constant. Historically, the biggest difficulties lie in establishing a reproducible phase boundary potential at the interface of the sensing membrane and the underlying electron conductor. This talk will address our newest finding using ion-selective membranes doped with redox buffers. It will also address the question on how water affects this phase boundary potential and how the time necessary for conditioning in aqueous sample solutions can be minimized.
Recently, we developed a label-free detection method of biomolecules using nanostructures [1-3]. This method is based on diffraction of the light of the nanostructures fabricated in a micro device. The refractive-index difference between the samples in the nanostructures and a device substrate brings about change of intensity of the diffracted laser beam. In this paper, we demonstrate a highly sensitive detection of DNA (48 kbp) molecules (detection limit, 0.18 molecules) using our label-free detection method.

Recently, extracellular vesicles have received researchers attention since the extracellular vesicles contain important cellular components such as RNA, DNA, and microRNA (miRNA). The miRNA analysis in the extracellular vesicles provides of great clinical importance as biomarkers for a variety of disease states. Several methods have been developed to isolate the extracellular vesicles from culture medium or body fluid; however, these methods require expensive facilities and large volume. In this study, we developed nanowire devices to isolate the extracellular vesicles from lower sample volume of culture medium or body fluid. We fabricated ZnO nanowires (100 nm in diameter and 2-3 µm in length) embedded in microchannels. The microchannels were etched using Reactive Ion Etching (RIE), and the ZnO nanowire were grown by aqueous synthesis in low temperature (85 °C for 20 hours). We compared extraction efficiency of miRNA in the extracellular vesicles using the nanowire devices with those using conventional methods. The conventional methods take over 24 hours at 4 °C by introducing agglutinating reagents to isolate the extracellular vesicles in the supernatant. In addition, the isolated vesicles were pelleted by centrifugation at 1500 g for 1 h. The pellets of the vesicles were re-suspended in lysis buffer to extract the miRNA. On the other hand, for nanowire devices, the miRNA could be extracted only by introducing lysis buffer after isolation of the vesicles inside the nanowire devices. Since the ZnO nanowires have positive charge on their surfaces, the negatively charged vesicles in the supernatant are trapped onto the ZnO nanowires by electrostatic interaction.

Keywords: Bioanalytical, Biotechnology, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Sampling and Sample Preparation
Liquid chromatography (LC) columns and column packing materials have been downsized to achieve the high-resolution separation of chemical compounds. These downsized tools prepare a high specific surface area, it causes not only high-resolution separation but high pressure resistance. Large pumps, that can generate high pressure without pump pulsation, are also required to achieve high resolution and fast separation. On the other hand, a miniaturized device for on-site analysis is required for tests of water quality, soil, quarantine and so on. It is necessary to reduce the pressure resistance of LC columns and develop micropumps for achieving the miniaturized LC device.

We have proposed a novel concept of the miniaturized LC columns. The LC columns have incorporated a pumping function based on an electroosmotic flow (EOF). Two kinds of LC columns have been developed to satisfy the criteria; one is capillaries with monolithic materials prepared by organic or inorganic synthesis, and the other one is microfluidic channels with microstructures made by microfabrication technologies. These both columns have a large specific surface area and high porosity. The large specific surface area has possibility to generate fast EOF and high pressure, and high porosity can probably reduce the pressure resistance.

Prepared silica monolithic capillaries have lower pressure resistance than polymer monolithic capillaries. The silica monolithic capillaries also generate sufficient pressure for liquids to flow into it. After the immobilization of silica surface with sulfonate, the immobilized silica capillaries have retention of the cationic solutes. As for microfluidic channels, the reduction of pressure resistance and retention of hydrophobic solutes were confirmed by designing microstructures. These columns can contribute to miniaturize LC devices for on-site analysis.

Keywords: Capillary Electrophoresis, Capillary LC, Lab-on-a-Chip/Microfluidics
Application Code: High-Throughput Chemical Analysis
Methodology Code: Liquid Chromatography
This paper reports the condensation and separation method of microdroplet contents by utilizing spontaneous emulsification nanodroplet formation at the microdroplet' interface. In this method, contents of the microdroplets were condensed, or separated depending on hydrophobicity and/or the size of the contents. For high throughput microdroplet-based analysis, versatile spectrometric detection is sometimes difficult to be applied because of its short optical path length. Another difficulty is a lack of in-line separation methods. As a challenge to solving these problems, we propose new condensation and separation method for microdroplets’ contents. When spontaneous emulsification occurs at the interface of microdroplets, the microdroplet shrink and nanodroplets are generated. If the contents stay in the microdroplet, the contents will be condensed in it. The condensation was demonstrated in a 100-[micro]m-deep 250-[micro]m-wide channel, where aqueous dispersed phase and organic continuous phase with excess surfactant (Span 80) were used. Then, condensation of tris (2,2′-bipyridyl)ruthenium chloride due to shrinkage of the microdroplets was observed. On the other hand, Fluorescein was partition to nanodroplets and was not condensed. Investigations on these condensation/separation process will be presented.

**Keywords:** Bioanalytical, Lab-on-a-Chip/Microfluidics, Microscopy

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Recently, microfluidics are further downscaling to 10-100 nm space, where nanofluidic field is formed. In this field, basic liquid properties are increasingly investigated, while device application is also developed for analysis and energy generation. For this purpose, detection method in this ultrasmall space is essential. In particular, absorptiometry is one of the popular detection method. However, due to the ultrashort pathlength (10-100 nm), the sensitivity was low. Our group developed a differential contrast thermal lens microscope, which detects heat after light absorption sensitively and realized detection in 50 nm space with zmol detectability. In this presentation, we introduce the novel detection method and the unique characteristics (UV absorptiometry by visible light) by combining with optical near-field.

Keywords: Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
The distribution of exocytotic activity has been found to be spatially heterogeneous at the surface of a single cell, resulting in hotspots where neurotransmitters are released more frequently. This subcellular heterogeneity across a single cell has thus motivated the design of microelectrode arrays (MEAs) capable of resolving the spatial variation of exocytosis across a single cell. The development of MEAs with electrodes small enough to allow quantitative measurement of released molecules from exocytotic hot spots distributed on the surface of a single cell is highly important to our understanding of the exocytosis process.

Here, we present the fabrication, characterization, and application of microwell-based MEA devices for high spatial resolution of release at single cells. The microwell-based MEAs consist of up to thirty-six 2-µm-width square ultra-microelectrodes, all inside a 40 µm × 40 µm SU-8 square microwell. The microwell is used for single cell trapping and single cell culturing on the surface of MEAs. Effective targeting and culturing of single cells in the microwell are achieved by these cell-sized microwell trapping and micropipette picking techniques. Imaging the spatial distribution of exocytosis at the surface of a single PC12 cell has been demonstrated with this system. Exocytotic signals from multiple independent ultra-microelectrodes at a single PC12 cell demonstrating the subcellular heterogeneity in single-cell exocytosis.

Keywords: Bioanalytical, Electrochemistry, Imaging, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Control in crystal shape and/or polymorphisms are essential issue in protein crystal structure analysis and material chemistry field. We found that crystal growth can be controlled in small droplet generated in microfluidic chip [1-3]. This technique is based on decreasing the supersaturation by controlling the protein concentration gradient in a microdroplet after the first nucleation. By this method, we could control generated crystal number within the droplet. First, we expand this approach to control the shape of protein crystal within small microfluidic chamber, because there is a demand on the controlling morphology and aggregation of protein crystals in order to obtain clear diffraction pattern via on-chip X-ray analysis. In particular, crystal habit of protein effects on the diffraction intensity. We found the potential of microfluidic chip for controlling crystal habit as well as aggregation of protein crystals by changing size and aspect ratio of microchamber on chip.

We also expand this approach to control polymorphism of organic materials. We employed L-glutamic acid, L-phenylalanine, indomethacin, paclobutrazol, as model compounds. In all cases, we were able to obtain the metastable form crystals by using simple droplet-based microfluidics, by decreasing supersaturation within droplet. These results demonstrate that microfluidic chip is a suitable apparatus for controlling crystal habit and polymorphism.

In order to establish partitioning methods for high level radioactive liquid wastes, various separation techniques such as solvent extraction and chromatography for actinide and lanthanide ions have been investigated. However, mutual separation of lanthanides (Ln) and actinides (Ac) is difficult, because these f block elements possess relatively similar physicochemical properties. Therefore, this study aims at investigating the adsorption mechanism of lanthanide trivalent ions (Ln(III)) by using non-crosslinked N-isopropylacrylamide (NIPAAm)-based polymers, where brush-like polymers immobilized on silica (SiO2) beads were employed.

The copolymerization of NIPAAm with N-vinyl-2-pyrrolidone (NVP) or 4-vinylpyridine (VPy) onto SiO2 beads was performed by using surface-initiated atom transfer radical polymerization (SI-ATRP). The thermo-responsive behavior of NIPAAm-based polymers was investigated for Ln(III). In less acidic aqueous solutions, all the NIPAAm-based polymers exhibited only adsorption behavior with regular selectivity (Eu3+>Sm3+>Nd3+>Ce3+>La3+) at below the phase-transition temperatures. This tendency suggests that hydrogen-bond network of Ln(III) hydration would also largely contribute to the adsorption mechanism in weak acidic solutions. On the other hand, in more acidic solutions, the NIPAAm-based polymers exhibited adsorption and desorption at below and above the phase-transition temperature, respectively. The absence of desorption phenomenon for VPy copolymers corresponds to the disappearance of LCST caused by the high protonation of pyridine N atoms. Based on the prospect that the entire hydrogen-bond strength is almost unchanged in the low pH region, hydrogen-bond network would not significantly contribute to the adsorption/desorption mechanism in strong acidic solutions. The adsorption mechanism depends on coordination of electron-donating groups in polymers to Ln(III) ions.

Keywords: Nuclear Analytical Applications, Polymers & Plastics, Separation Sciences, Surface Analysis
Application Code: High-Throughput Chemical Analysis
Methodology Code: Separation Sciences
Our novel infrared imaging spectroscopy technology, introduced for the first time at Pittcon 2014, is equipped with an interferometer where interference takes place only when split light beams are combined again on the focal plane. There are two types of implementation of this technology, 2D type and one-shot type devices. The 2D type device is particularly suitable to take a spectroscopic image of as wide an area as a few 10 centimeters square like a human face.

We implemented the 2D type interferometer in as small device as a 50mm cube. This newly developed device is more than five times smaller than the previous one. Such a small device is suitable for several point-of-sales applications one of which is the whole face skin spectroscopy for the purpose of skin care.

Moisturizing cosmetics are used to help retain skin tension and elasticity. The amount of water contained in facial skin can be a good parameter to check how these agents work well and could help the customer choose the most appropriate moisturizing cosmetics.

When coupled with a compact 100 x 100 pixel infrared detector array, this device can divide the whole face into 100 x 100 pixels and infrared spectra are obtained for each of the pixels which could help estimate the facial skin moisture distribution.

This study was supported by a grant from the Japan Science and Technology Agency.
Bio Applications of Vibrational Spectroscopy

Surface Plasmon Resonances in the Mid-Infrared

Over the past several years, significant interest has been aimed towards determining the refractive index of living cells. By monitoring the refractive index important biological information can be assessed including cell activation, viability, accumulation and local interaction with biological stimuli. Current techniques to determine the refractive index of living cells are limited due to the non-uniformity of the cellular matrix and poor cellular adhesion. Surface Plasmon Resonance (SPR) has successfully been applied to refractive index monitoring of cellular cultures. SPR is advantageous for this type of detection because it is highly sensitive to small changes in the refractive index and provides a real-time response to changes in the cellular matrix. Previous investigations with SPR into the refractive index of living cells have been confined primarily to the visible and near infrared regimes. In these regimes, the penetration depth of the SP into the media is small, on the order of a few microns, whereas cellular height can vary from 2-12 μm. By extending SPR further into the mid-infrared, larger tunable penetration depths can be achieved which are compatible with the size of living cells. Herein we expand upon current MIR-SPR methodology, discuss the plasmonic resonances and characterize the bulk and surface sensitivities for gold in the MIR.

Keywords: Analysis, Detection, FTIR, Spectrophotometry
Application Code: General Interest
Methodology Code: Vibrational Spectroscopy
In this article we report the maillard reaction, which was well researched by food, environmental, and organic chemistry field was induced inside human hair and analyzed micro changes by using focal plane array (FPA) detector attached to FT-IR. Arginine(A), glycerin(G), and xylose(X) which are common food ingredients were used to generate the maillard reaction. Each ingredient were dissolved in purified water and then heated at 150°C to induce maillard reaction. In this process label free study of monomer, which is the generated substance during maillard reaction kinetics, was proved. And the monomer structure was determined by using nuclear magnetic resonance (NMR) and FT-IR. The monomer formed within human hair was found by using FPA and it is the world first report.

**Keywords:** Analysis, Chemical, FTIR, NMR

**Application Code:** Bioanalytical

**Methodology Code:** Vibrational Spectroscopy
Bio Applications of Vibrational Spectroscopy

UV Resonance Raman Studies of Primary Amide Vibrations

UV resonance Raman (UVRR) spectroscopy has been applied to monitor the dependence of primary amide vibrations on hydration and conformation. Excitation within the electronic transition of the primary amide group selectively enhances primary amide vibrations. We obtained UVRR spectra of propanamide in solution and in the solid state in order to investigate the dependence of primary amide vibrations on hydration. The primary amide I, II, and III, and NH2 rocking vibrations all display significant sensitivity to hydrogen bonding. We measured the Raman cross sections of the primary amide bands of propanamide in water and used these to determine the changes in cross section as a function of increasing mole fraction of water. The primary amide Raman band frequencies can also be related to the primary amide hydration. We will discuss these relationships and how they can be applied to molecules containing primary amides to determine the environment of the primary amide group. Additionally, we obtained visible Raman, IR, and pre-resonance Raman spectra of propanamide, butyramide, valeramide, L-glutamine, L-asparagine, and N-acetyl-L-glutamine in order to probe the dependence of primary amide vibrations on conformation. Preliminary results indicate that the primary amide NH2 rocking vibration, which is enhanced in the pre-resonance Raman spectra, is sensitive to the O=CCC torsion angle. This vibration may be used to determine the O=CCC torsion angle distribution of side chains that contain a primary amide group, e.g., glutamine, in peptide and protein structural studies. Funding for this work was generously provided by the University of Pittsburgh and NIH 5 T32 GM 88119-3 (EMD).

Keywords: Bioanalytical, Biospectroscopy, Infrared and Raman, Vibrational Spectroscopy
Application Code: Bioanalytical
Methodology Code: Biospectroscopy
A recent study [1] has shown that ATR-FTIR, coupled with support vector machines, can discriminate low and high grade glioma (brain cancers) serum from serum collected from healthy patients. Highlighting the potential of IR spectroscopy for the screening of blood serum, which has the potential for significantly reducing current diagnosis times and greatly increasing the chance of successful treatment. However, the current instrumental setup only allows sequential data collection with each spectral acquisition of a blood serum spot taking approximately 10 minutes when taking into account drying times.

Recently, the first commercial laser based infrared micro-spectrometer has been developed by Daylight Solutions Inc. The Spero instrument incorporates a broadly tunable quantum cascade laser source producing hyperspectral images with a wide field of view, micron spatial resolution and high signal to noise over the 900-1800 cm⁻¹ spectral range; hyperspectral images being recorded in 12 minutes over the full 900-1800 cm⁻¹ spectral range. The Spero also has the unique ability of discrete sparse frequency imaging of samples, recording absorption information from a number of pre-selected wavelengths, further increasing the speed of spectral image acquisition times. We present the use of Spero for the rapid screening of cancerous blood serum, recording spectral images over the full achievable spectral range as well as discrete frequencies, chosen based on their saliency for distinguishing normal from cancerous blood serum. With sparse images recorded based on 12-14 discrete wavenumber frequencies being approximately 6 times faster than spectral images recorded over the full achievable range, sparse spectral images produced have the potential for the rapid diagnosis of blood serum. We also discuss recent methodological developments for high throughput serum analysis and diagnosis.


Keywords: Bioanalytical, Biomedical, Biospectroscopy, Infrared and Raman
Application Code: Biomedical
Methodology Code: Biospectroscopy
Detecting and studying chemical changes on the surface of implanted medical devices during infection is challenging because the chemical sensor must provide a low background, surface-specific signal that can pass through tissue. We are developing sensor films that can be coated on implanted devices and interrogated through tissue with a combination of X-ray excitation and visible spectroscopy. When an X-ray beam irradiates the implant surface, light is generated by X-ray scintillators coated on the implant. This light first passes through a chemical sensor layer (e.g. pH indicator-loaded film that alters the spectrum according to pH), then passes through the tissue, and finally is detected by a spectrometer. A reference region on the implant is used to account for tissue-induced spectral distortion. pH images are acquired by moving the sample and collecting a spectrum at each location, with a spatial resolution limited by the X-ray beam width. Using this technique, we detected a pH drop during normal bacterial growth on the sensor surface, and a pH restoration during antibiotic treatment through with millimeter resolution through 6 mm ex vivo porcine tissue. We can also detect other spectrochemical changes such as dissolution of silver nanoparticles coated on an implant surface. Overall, this technique enables noninvasive, high-spatial resolution chemical imaging of implant surfaces through tissue to detect and study implant infection.

**Keywords:** Luminescence, Method Development, Microscopy, UV-VIS Absorbance/Luminescence

**Application Code:** Bioanalytical

**Methodology Code:** Biospectroscopy
FTIR spectrochemical images of Arctic sea ice diatoms have been obtained at a nominal 1.1 µm pixel edge spatial resolution, using upgraded optics installed in our thermal-source Agilent Cary 620 microscope (64x64 Focal Plane Array) attached to an Agilent Cary 670 spectrometer. The new optics provide the capability for diffraction-limited spatial resolution and thus enable bench-top FTIR spectromicrotomographic (SMT) imaging, previously demonstrated only with a multi-beam synchrotron-based system (Martin et al, Nat. Meth. 2013 10:861-864). As diatoms are a major primary producer in the Arctic spring, the volume of organic matter in organelles contained within their encapsulating silica frustules is relevant to the health of these organisms, and to the Arctic ecosystem at large. We have now used FTIR SMT to evaluate the composition of individual, intact diatoms, harvested from a landfast first-year ice site in Resolute Passage, NU, Canada (74 43.628’N; 95 33.330’W) and from Dease Straight, an important section of the Northwest Passage. Diatoms, selected individually under a dissecting microscope for species-specific examination, were captured on a MiTeGen polyamide microloop, to be mounted onto a goniometer and imaged. No further treatment of the specimen was required, as only native signals from unaltered diatoms are measured, and these are well resolved spectrally. Data import, computed tomography (CT) and voxelated display algorithms in MatLabᵀᴹ were adapted for back-projection of FTIR images. The distribution and abundance of compounds was reconstructed while maintaining a sub-cellular level of spatial resolution in three dimensions.

Keywords: Biospectroscopy, FTIR, Imaging, Microspectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
The persistent need for biological and diagnostic tools has resulted in a growing demand for targeted affinity reagents. While antibodies remain the gold standard for affinity interactions, aptamers—short, single-stranded oligonucleotides that bind target molecules with high affinity and specificity—have emerged as a viable alternative. Aptamers derive their functionality from the various secondary conformations they adopt, including the G-quadruplex (G4) structure. The G4 structural motif is prevalent throughout the human genome, particularly within oncogene promoter regions, and may play an important regulatory role in gene transcription. Thus, guanine-rich promoter regions represent a source for potential aptamer sequences as well as possible targets for oncogene regulation. We have previously proposed a genome-inspired reverse selection pathway towards aptamer discovery based upon naturally occurring DNA sequences within human oncogene promoter regions. Here, we describe the application of our method to investigate several oncogenic promoters, including c-myc and c-myb, and their interactions with proteins extracted from the nuclei of human cancer and tumor cells. We report our findings concerning the captured proteins from initial screening by MALDI-TOF MS through sequence analysis via LC-MS/MS. In this way, new affinity interactions can be discovered, offering further insight into aptamer behavior as well as potentially elucidating biomarkers that may aid in cancer diagnosis and treatment.
Abstract Text
Vitamin D comprises a class of lipid-soluble secosteroids responsible for regulation of calcium stores in the human body. Deficiency can lead to osteomalacia, and has been implicated in cancer, cardiovascular disease, and diabetes. Immunoassays and LC-MS/MS are used to quantitate 25-hydroxy vitamin D2 (25-OHD2) and D3 (25-OHD3), the most common serum biomarkers. However, serum also contains a significant concentration of their less potent respective C3 epimers, which can interfere with accurate quantitation. Although LC-MS/MS methods capable of chromatographically resolving these epimers have been developed, ion mobility spectrometry (IMS) offers an additional dimension of separation for the epimers.

We have evaluated two mobility strategies: drift tube IMS and field asymmetric IMS (FAIMS). Drift tube IMS separates compounds based on differences in mobility (collision cross section) through a drift tube. FAIMS separates compounds based on differences in their high and low field mobility. While 25-OHD2 (m/z 412.6) and 25-OHD3 (m/z 400.6) differ in molecular weight and can be separated using mass spectrometry, their respective C3 epimers have the same molecular weight and fragmentation patterns, and thus cannot be differentiated. Preliminary experiments using an Agilent 6560 IMS quadrupole time-of-flight mass spectrometer (IMS-QTOF-MS) show that sodiated 25-OHD2 and 25-OHD3 can be separated, with collision cross sections of 206.8 and 202.0 Å[sup]2[/sup], respectively. Studies are underway to determine optimal separation conditions for the C3 epimer compounds. FAIMS is also capable of separating low molecular weight isomers, especially with the use of modifier gases. Studies are underway to optimize drift gas (e.g., nitrogen, carbon dioxide) and modifiers, such as solvent vapor, for separation of the C3 epimers with IMS-MS/MS and FAIMS-MS/MS. These techniques offer an additional opportunity for separation of isomeric metabolites in complex matrices using LC-MS/MS.
In the present study, Time of Flight Secondary Ion mass spectrometry (ToF-SIMS) was used to investigate the lipid localization in brain and intestine tissue sections from rats fed specially processed cereals and control rats fed ordinary feed. Intake of the processed cereals increase active antisecretory factor in plasma, an endogenous protein with proven regulatory function on inflammation and fluid secretion. This endogenous increase counteracts the clinical intestinal symptoms in patients suffering from inflammatory bowel disease is also known to provide protection against raised intracranial pressure induced by experimental head trauma.

An IonTof 5 instrument equipped with a Bi cluster ion gun was used to analyze the tissue sections. Data from 15 brain and 15 intestine samples from control and activated AF rats were recorded using the stage scan macro raster with a lateral resolution of 5 μm. Data were subsequently exported to MatLab and then subjected to principal components analysis (PCA). The preliminary data clearly showed changes of certain lipids following activation of aAF from SPC cereal feeding. In the brain tissue samples, PCA score plots showed a good separation in lipid distribution between the control and the SPC-fed group. The loadings plot revealed that the groups separated mainly due to changes in cholesterol, vitamin E and c18:2, c16:0 fatty acid distribution as well as some short chain monocarboxylic fatty acid compositions. PCA score plots from intestine sections also showed a well-defined separation between the control and the aAF group. Here, the c18:2, c18:1, c16:0 as well as the distribution of several other short chain monocarboxylic fatty acids were changed between the two groups. Hence ToF-SIMS has provided important insight into the working mechanism of a factor known to activate aAF and provided clues for a subsequent targeted quantitative analysis of the lipid content in brain and intestine.

Keywords: Bioanalytical, Imaging, Lipids, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Bacterial biofilms, such as those created by the gram-negative bacterium [i]Pseudomonas aeruginosa[/i], have a rich, three-dimensional spatial and chemical architecture that allows a clonal consortium of single cell organisms to live as a cohesive unit. Biofilm colonies thrive in a variety of environments, including on the surface of fuel tanks, in the depths of our lungs, and at the junctions of human prosthetics. We combine polyatomic secondary ion mass spectrometry (SIMS), matrix assisted laser desorption ionization (MALDI), and electron microscopy (EM) to develop a multimodal analytical approach capable of exploring the complex heterogeneity contained within bacterial communities. Using a chemical inkjet printer, a micro-droplet array of MALDI matrix is applied directly to the surface of [i]P. aeruginosa[/i] biofilms. The spots in this array are subsequently used for both MALDI analysis, which provides information on the distribution of high mass biomolecules, and as fiducial markers for sample navigation and image correlation. The area between the matrix spots is then subjected to bombardment with a non-destructive C[sub]60[/sub][sup]+[/sup] ion beam, yielding molecular ion images at high spatial resolution. The film is finally imaged with EM, providing structural information corresponding to the chemical images acquired by SIMS and MALDI. Initial SIMS results show micron scale chemical heterogeneity including areas with high levels of specific cell-cell signaling molecules (e.g. quinolones HHQ, NHQ and C[sub]9[/sub]-PQS), while MALDI reveals more homogenous distributions of several larger biomolecules at macroscopic scale. Funding provided by the National Institutes of Health, the Department of Energy and the Springborn Endowment.
Human histone deacetylase 8 (HDAC8) serves as an essential epigenetic regulatory enzyme in gene transcription. HDACs act as components within many multi-protein complexes, and bind to metal ions in vivo. Understanding how HDAC8 interacts with other proteins, and the effects of metal binding on its enzymatic activity, are long-standing challenges for structural and molecular biology. Ion mobility-mass spectrometry (IM-MS) is an information-rich assay for protein-protein complexes, as it enables structure assessments on complexes from small amounts of sample within heterogeneous mixtures. Herein, Poly-r(C)-binding protein 1 (PCBP1), an iron chaperone, is studied as a potential binding partner for HDAC8 using IM-MS. Specifically, we investigate the interaction, stoichiometry, metal binding, and structure of the complex. HDAC8 has one metal (e.g. Zn) binding site, and PCBP1 has three iron binding sites. Previous work suggests that HDAC8 interacts with PCBP1, but does not ascribe specificity the complex. Our nESI-IM-MS data, however, indicates the two proteins bind specifically, forming a heterodimer at low protein concentrations in vitro to an extent that appears enhanced relative to any homodimers. The specificity of the HDAC8-PCBP1 heterodimer was tested further by introducing Carbonic Anhydrase II (CAII) as a negative control. No significant ion intensity was detected for CAII hetero-complexes in our IM-MS experiments, further confirming the specificity of the HDAC8-PCBP1 hetero-dimer. To further investigate the role of metals on the structure of the complex, samples containing either apo, zinc or iron(II) bound HDAC8 were incubated with apo, as well as iron(II) bound PCBP1 and screened for complex formation and structural effects by IM-MS. This presentation will contain our latest IM-MS results that track the binding efficiencies and structures of these newly-discovered, centrally-important protein complexes.
Proton-transfer-reaction-time-of-flight-mass-spectrometry (PTR-ToF-MS) is a powerful tool for real-time analysis of VOC profiles in breath. Time resolutions as low as 200 msec and detection limits in the pptV-ppbV range enable breath-resolved measurements in mechanically ventilated and spontaneously breathing patients.

The high water content in breath samples however can have a distinct impact on the quantification. The objective of this study was therefore to systematically investigate the effects of humidity on the quantitative detection of breath VOCs and the adequacy of standard data processing algorithms applied in PTR-MS.

More than 20 VOCs (hydrocarbons, ketones, aldehydes and aromatics) were investigated in different trace concentrations (2, 5, 10, 25, 50 and 100 ppbV) and different amounts of humidity (0, 1, 25, 50 and 100% rel. humidity). Further, normalization of data onto primary ion count and normalization onto primary ion count in combination with watercluster ion count was tested.

Measured intensities of VOCs varied up to 40% when water content was varied, while variation was less than 5% when water content was kept constant, independent of the total amount of water in the sample. Normalization onto primary ions reduced the variation for aldehydes (from 40 to 32% for heptanal e.g.), but increased it for aromatics (from 8 to 15% for toluene). These effects were even more pronounced when watercluster was included in the normalization process. Quantification of biomarkers thus can be compromised if matrix effects like humidity are neglected. Hence, adapted calibration mixtures are mandatory for the reliable quantification of humid samples in real-time PTR-ToF-MS measurements.

**Keywords:** Bioanalytical, Biomedical, Mass Spectrometry, On-line

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
Surfactants are used in protein sample preparation and analysis to improve protein solubility and stability. Sodium dodecyl sulfate (SDS) is a common anionic surfactant which is currently used for protein analysis by Matrix Assisted Laser Desorption Ionization (MALDI) - Mass Spectrometry (MS). The major drawbacks of SDS use in MALDI-MS protein analysis are deleterious spectral effects which are caused by sodium-protein adducts formation. The use of SDS affects the resolution and signal to noise ratio which results in inaccurate mass analysis. We have synthesized novel cationic ionic liquid surfactants (ILS) which can be used as additives in protein samples for MALDI-MS analysis. The spectral effects in protein analysis of both ILS and SDS were examined and compared at different concentrations (0%, 0.02%, 0.05%, 0.1%, 0.5%, 2%, and 5% (w/v)) using a MALDI-TOF mass spectrometer. Interestingly, the protein signal intensity was significantly improved in the presence of low concentrations of ILS as compared to 0% ILS. Above the concentration of 0.5% ILS, the signal intensity was reduced. Conversely, the protein signal was suppressed at low concentrations of SDS as compared to 0% SDS and the signal was highly degraded at 0.1% SDS. Recovery of signal intensity occurred above the concentration of 0.1% SDS. The results suggests that low concentrations of cationic ILS can be used as MALDI MS signal resolving agents for protein analysis where high concentrations of anionic SDS are currently required to obtain comparable spectral features.

The work is supported by the National Science Foundation under Grant number CHE-1307611.
Discovery of biological markers at the earliest possible stage of cancer progression is a priority in all aspects of cancer-related research. Earlier detection methods and more accurate markers result in lower mortality rates and higher quality of life for patients. Recent studies have been published revealing an apparent difference between the volatile metabolome of melanoma and melanocyte cells, but only after preconcentration and preparation steps. Identification of additional biomarkers, as well as development of a real-time method incorporating little or no sample preparation would revolutionize current melanoma-detection schemes.

Three mass-spectrometric setups were utilized: an Agilent 6220 TOF, a Thermo Scientific LTQ XL, and a Thermo Scientific Q Exactive. Multivariate data analysis was performed through MetaboAnalyst and SIEVE. Cells of a given type were pooled in H$_2$O, lysed in cold 80% MeOH/H$_2$O to precipitate proteins, and then centrifuged before drying the supernatant under nitrogen at 35 [degree]C. Dried samples were reconstituted with 0.1% FA in H$_2$O for LC/HRMS or 80% MeOH/H$_2$O for direct analysis (APCI/MS and DART/MS).

In preliminary studies, normal melanocyte and malignant melanoma human cell cultures were analyzed by LC/HESI/HRMS, APCI/MS, and DART/MS. Both PCA and PLSDA were performed on the data, and first component separation was ubiquitous among all methods. This finding suggests a distinct difference between the volatile and semi-volatile metabolomes of melanoma cells vs normal melanocytes, in addition to allowing identification of potential biomarkers for melanoma. Ongoing experiments have been performed on swabs of live human skin at sites of normal skin and latent melanomas.

Keywords: Bioanalytical, Clinical Chemistry, Detection, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Accurately detecting and de-convoluting overlapped chromatographic peaks generated by co-eluted metabolites in GC-MS is the key to high accuracy metabolite identification and quantification. We developed an algorithm for de-convoluting the overlapped chromatographic peaks at the mass spectrum level, including baseline correction, total ion current (TIC)-based peak detection, selected ion chromatogram (XIC)-based curve fitting, and chromatographic peak center and width–based clustering. The baseline correction removes background noise for accurate determination of true peak shape from the TIC and XIC, respectively. We used TIC-based peak detection to recognize candidate chromatographic peak ranges followed by Gaussian model-based curve fitting. In order to determine the number of compounds present in a range of overlapped chromatographic peaks, mass spectrum similarity scores between every two adjacent spectra were calculated. The number of co-eluting compounds was considered as the initial number of Gaussian functions. A mixed Gaussian model was then employed to fit each XIC by minimizing the curve fitting error. After the Gaussian model fitting, the XIC of each compound is represented by a set of coefficients, peak center and peak width. We further use pairs of peak center and peak width to distinguish the co-eluted compounds by retention time.

Our developed method was applied to analyze GC-MS data of mixtures of standard compounds. Compared with the software from the Agilent instrument, our method found more compound standards with high values of positive predictive value, true positive rate and their F1 score.

**Keywords:** Bioinformatics, Detection, Gas Chromatography/Mass Spectrometry, Statistical Data Analysis

**Application Code:** Bioanalytical

**Methodology Code:** Chemometrics
USEPA, Office of Groundwater and Drinking Water, and CB&I Federal Services LLC have developed an improved method for estimating the lowest concentration at which ‘reliable’ measurements of analyte concentration can be made, known as the Lowest Concentration Minimum Reporting Level (LCMRL). The LCMRL procedure is a single laboratory, single analyst method. It is currently used by individual laboratories to establish their lowest achievable reporting limit for Drinking Water analysis.

The LCMRL is the lowest concentration for which future sample recovery is predicted to be between 50 and 150%, with high confidence (99%). The procedure simultaneously takes into account precision and bias. A designed experiment is carried out in which samples at each of several known concentrations are taken through the entire measurement process. Regression functions for the mean and variance of the measured concentration are estimated from this experimental data. The variance function is a constant + power function, which includes the well-known Horwitz Law for the variance of repeated analyses as a special case.

Given the estimated mean and variance functions, the distribution of repeated measurements at a specified concentration is taken to be Gaussian when negative measurements are possible (such as when background or ‘dark current’ corrections are required) or Gamma when they are not. Given this information, we can estimate conditional probabilities of any event that we are interested in, including the probability that the measured value is between 50% and 150% of the true value. The lowest concentration at which this coverage probability equals the desired confidence (99%) is the LCMRL.

**Keywords:** Chemometrics, Environmental Analysis, Trace Analysis, Water

**Application Code:** Environmental

**Methodology Code:** Chemometrics
Anomaly and target detection methods tend to be treated as independent algorithms for analysis of hyperspectral images. The difference in the approaches is that target detection is a supervised pattern recognition technique while anomaly detection is an exploratory analysis method. Target detection algorithms such as generalized least squares (a.k.a. matched filter) typically employ known, well-characterized, library spectra for detection and classification. Unfortunately, due to measurement related differences between the library and the measured data (e.g., matrix effects) there may be mismatch between the library and the measured spectra resulting in a compromise in detection performance. Additionally, only the selected target is considered in the detection task. On the other hand, anomaly detection methods such as maximum signal factors (MSF) can be used with current measurements to explore anomalies where the observed anomaly is used as the measurement relevant target. Recent advances in MSF algorithms have improved detection performance, reduced memory and time requirements, and reduced the number of scores images that to be inspected during exploratory analysis. However, the detected anomaly must be compared to library spectra for classification. As a result, it should be seen that target detection and MSF form complimentary approaches to detection and classification resulting in measurement relevant target detection. Examples of the two approaches, applied separately and together, will be shown with hyperspectral images.
The large amount of data generated by modern analytical instruments and the complexity of samples being analyzed is fueling interest in the use of chemometric techniques. However, with the inordinately large number of variables per sample present in some data sets (notably chromatography-mass spectrometry) some form of feature selection is mandated. This is due to the presence of a large number of irrelevant variables which will collectively degrade or completely obscure any useful signals in the data. We have previously developed and demonstrated a model quality assessment parameter for classification models termed Cluster Resolution (CR), which lends itself to guiding automated feature selection processes.

Generally, the CR metric is used in a hybrid backward elimination / forward selection algorithm for objective variable subset selection in supervised classification/pattern recognition models. In earlier works, CR-FS was applied to classification of complex samples with a high degree of success. Notwithstanding, the performance of this algorithm has not been compared with other feature selection techniques already available.

In this study, we benchmark CR-guided feature selection against other feature selection algorithms such as genetic algorithm, random forest, etc. The parameters for objective comparison include feature selection time; model quality i.e. specificity, sensitivity and accuracy; robustness of the FS process and the chemical significance of the features that survive the process.
Pattern Recognition Assisted Infrared Library Searching of the PDQ Database to Improve Investigative Lead Information from Automotive Paints

Modern automotive paints have a thin color coat which on a microscopic fragment is often too thin to obtain reliable chemical information. The small size of the fragment also makes it difficult to compare it with manufacturer’s paint color standards. Since adhesion between paint layers is strong, both primer layers are often transferred during a collision if the clear coat and color coat layers are also transferred. As the clear coat and primer layers are often unique to the automotive assembly plant where these layers are applied, combining chemical information obtained from IR spectra of the two primer layers and from the clear coat layer has made it possible to rapidly and accurately identify the assembly plant of an automobile from its paint system. Applying data fusion techniques where IR spectra from multiple sources (e.g., the clear coat and primer paint layers) are combined and class membership information is extracted, search prefilters for the PDQ library have been developed to determine both the manufacturer and the model of a vehicle from an automotive paint sample. Even in challenging trials using a database of 1324 paint samples where the clear coat and undercoat paint layers evaluated were from GM, Ford, and Chrysler with a limited production year range (2000-2006), the respective assembly plant of an automobile was correctly identified using only information extracted from IR spectra of the clear coat and primer paint layers. Applying wavelets to preprocess the IR spectra, advantages associated with higher order data fusion techniques were achieved when a genetic algorithm was used to identify the informative wavelet coefficients. The development of search prefilters to cull library spectra to a specific assembly plant and a cross correlation library searching algorithm to identify spectra most similar to the unknown in the set of spectra identified by the search prefilters is the subject of this presentation.

Keywords: Chemometrics, Forensics, Infrared and Raman, Informatics
Application Code: Homeland Security/Forensics
Methodology Code: Chemometrics
The ability to rapidly generate chemical-based image contrast from hyperspectral image data is a fundamental challenge due to the large quantity of data in these datasets. Currently-used spectral angle-based methods can be problematic because obtaining the same correlation scores for two distinct spectra becomes probable as the number of spectra increases or as the signal-to-noise ratio decreases. We have developed a fully-automated method called reduction of spectral angle symmetry (ROSAS), which is effective in differentiating spectral shapes in hyperspectral image data. ROSAS is a data reduction method that enables estimates of the original spectra to be recovered from the smaller-dimensioned sets of cosine scores. The effectiveness of the recovery algorithm can be calculated based on the magnitude of the resulting spectral vector. By utilizing principal component analysis with cosine correlation analysis, chemical-based image contrast is generated that results in the reduction of degeneracies caused by angular symmetries. ROSAS can be done rapidly, requires no training datasets or a priori information about the sample, and takes advantage of the full color-space of modern RGB displays. A full theoretical description of the method is given along with performance metrics. The description has been generalized to work with any number of wavelength dimensions and spectra. A concise protocol is put forth that will enable other researchers to utilize this method by following a short, simple list of steps.

Keywords: Biospectroscopy, Chemometrics, Data Analysis, Imaging
Application Code: Bioanalytical
Methodology Code: Chemometrics
A novel application of linear discriminant analysis (LDA) is proposed for determination of optimal digital filters for data deconvolution. The theoretical framework underpinning the generation of LDA-filters is described. Results from the use of LDA-filters are presented for recovering impulse responses in photon-counting from a high speed photodetector (rise time of \(~1\) ns) and applied to remove ringing distortions from impedance mismatch in multi-photon fluorescence microscopy. Training of the digital filter was achieved by defining temporally coincident and non-coincident transients, and identifying the projection within a high-dimensional "filter-space" that best separated the two classes. Once trained, data analysis by digital filtering can be performed quickly. Assessment of the reliability of the approach was performed through comparisons of simulated voltage transients, in which the ground truth results were known a priori. The LDA filter was also found to recover deconvolved impulses for single photon counting from highly distorted ringing waveforms from an impedance mismatched photomultiplier tube. The LDA filter was successful in removing these ringing distortions from two-photon excited fluorescence micrographs and from data simulations was found to extend the dynamic range of photon counting by approximately three orders of magnitude through minimization of detector paralysis.

Keywords: Chemometrics, Fluorescence, Microscopy
Application Code: Biomedical
Methodology Code: Chemometrics
How does one compute the mathematical certainty of wavelength standards used for calibrating instrumentation used for molecular spectroscopy measurements? This question becomes of major importance when the technique used for measurement requires collection of large databases for use in qualitative searches or quantitative multivariate analysis for multiple applications. Wavelength drift over time within a single instrument or wavelength differences between instruments creates errors and variation in the accuracy of measurements using databases collected with different wavelength registrations. The loss of integrity over the wavelength axis of data collected over time is a significant problem in large database creation and usage. So what are the techniques and mathematics used to compute uncertainty, and the optimum methods for maintaining wavelength accuracy within instrumentation over time, when considering measurement condition changes? This paper addresses a comparison of commercial instruments for wavelength precision and accuracy and what techniques are available for improving the agreement between first principles physics and metrology as compared to current common practices (1-2).

References:

Keywords: Chemometrics, Infrared and Raman, Near Infrared, Statistical Data Analysis
Application Code: General Interest
Methodology Code: Chemometrics
Chromatography in Fuels, Energy and Petrochemical Analyses

Bonded High Retentive and Selective Column for Analyzing Hydrocarbons, Sulfur Gases and Halogenated Compounds

In general PLOT columns are used for analyzing volatile compounds. Alumina has an ideal selectivity for light hydrocarbons, but it has a limitation for many other compounds. Also water in the sample or carrier gas, has a big impact on retention reproducibility.

Using Silica as an adsorbent, a much wider range of components can be analyzed.

Besides hydrocarbons, silica also elutes many other neutral compounds like halogenates, and sulfurs. Even slightly polar compounds like ketones or aldehyde elute form silica surface. Additionally, by using surface deactivation, one can make silica also in such a way, that water does not impact retention.

The challenge is to create silica surface that has enough retention and loadability to be used as a GC PLOT column. A new adsorbent material has been introduced called Silica BOND, which provides a high selectivity for hydrocarbons without adsorption of moisture. The new PLOT column also shows a high degree of inertness as it elutes also polar volatile compounds.

Besides this, a new immobilization process is developed making the column suitable for valve switching and high flow applications. Several applications of this new adsorbent will be presented and discussed.

Abstract Text

Keywords: Capillary GC, Gas Chromatography, Petrochemical
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography
Chromatography in Fuels, Energy and Petrochemical Analyses

Innovative Optical GC System for Municipal Waste Gasification and Biorefining Processes

Thermochem Recovery Intl (TRI Inc) is developing integrated thermochemical biorefineries that have recently begun demonstrating conversion of municipal solid waste (MSW) to Fischer Tropsch fuels. By undertaking the challenge of converting MSW to a gas that must then be refined and used for synthesis, TRI has found it absolutely necessary to improve on traditional methods of refinery gas analysis.

MSW syngas contains an unpredictable and extremely large range of organic and inorganic species rarely seen in other gasification and refining developments. To allow further refining of this syngas, near real time analysis with ppb level sensitivity for numerous organic and inorganics sources of sulfur, nitrogen, and chlorine is a necessity. Traditional methods like GC-FID, GC-TCD, GC-SCD, and GC-MS are not functional due to dynamic gas compositions and/or lack of qualitative or quantitative capabilities. Maintaining suitable calibrations for the wide range of compounds and concentrations is also problematic.

After years of collaboration with Prism Analytical on various gas analyses, TRI is excited to report on a new technology that offers a paradigm changing analytical solution for this and other refining applications. GC-MAX™ provides near real time analysis of MSW syngas regardless of the potential interferences. From the analytical perspective MSW syngas is a very challenging gas stream to analyze both qualitatively and quantitatively. Despite this complexity, initial tests with the GC-MAX™ technology on model MSW syngas suggest that it can provide the analysis and speeds required for tight process control and optimization. The GC-MAX™ technology and the MSW syngas application will be discussed.

Keywords: Fuels\Energy\Petrochemical, On-line, Process Analytical Chemistry, Volatile Organic Compounds
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Process Analytical Techniques
Wood is one of the possible starting materials for producing biofuels. Unlike crop sources for biofuels (e.g. corn and sugar cane), wood can be harvested year round. In order to produce a biofuel from wood, it must first be broken down to its fermentable carbohydrates. This is often accomplished by acid hydrolysis. Fermentation then yields the biofuel (e.g. bioethanol). To maximize process efficiency it is necessary to determine the types and amounts of fermentable carbohydrates in the wood acid hydrolysate. We designed a high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) method to separate and quantify the neutral sugars fucose, arabinose, galactose, glucose, xylose, mannose, fructose, cellobiose, and maltose contents of wood acid hydrolysates. Rhamnose content can be determined with the same method by lowering the column temperature from 45 to 30 oC. To determine the galacturonic and glucuronic acid contents we designed a method using another HPAE column. These methods yield a nearly comprehensive assessment of the carbohydrates present in the wood acid hydrolysate. Another method, using a third HPAE column, was developed to determine the neutral sugars and uronic acids in a single method, albeit in a longer time than required for the other two methods combined. The utility of these methods was demonstrated by quantifying the carbohydrates in a Loblolly pine wood acid hydrolysate and a rinsate.

Keywords: Biofuels, Carbohydrates, Method Development, Paper/Pulp
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Liquid Chromatography
High quality control of hydrogen gas, during production, transportation, storage, and utilization, is requested for commercialization of hydrogen and fuel cell technology. With the aim of assuring uniformity in the final quality of the hydrogen product, the International Organization for Standardization has been considering an international standard of technical specification, indicating the maximum content of certain non-hydrogen gases (ISO 14687-2). The technical issues being addressed in this standard, which has been published in December 2012, concern the detection of impurity species in hydrogen, at levels that are for some of them very low (ppb level). Especially, achieving the detection of active sulphur compounds at ppb-level, the major poisons of fuel cells systems, is highly challenging. Considering the ISO requirements, the Analytical Sciences Group at Air Liquide has developed a series of accurate and robust analytical methods using a single, multifunction instrument based on gas chromatography coupled to different detection systems. The instrument, which allows the detection of carbon monoxide, nitrogen, oxygen, argon, methane, carbon dioxide, but also non-methane hydrocarbons, sulphur compounds and formaldehyde, will be described.
The Aviv Analytical 5975-SMB GC-MS with Cold EI provides significantly enhanced molecular ions for all hydrocarbons and thus enables their vastly improved analysis including their isomer distributions. In addition, isomers and structurally related fragments mass spectral peaks are exhibited which enable their NIST library identification. Furthermore, extended range of compounds are amenable for analysis with short column and high column flow rates, including low volatility large oil and wax compounds that cannot be analyzed by standard GC-MS. In addition, the 5975-SMB GC-MS enables much shorter analysis time and improved sensitivity that could be up to and over three orders of magnitude better on the molecular ions of large hydrocarbons than what can be achieved with standard GC-MS.

The isomeric content and its distribution affect all the major physical and chemical properties of oils and fuels, including: combustion efficiency, octane number, flash point, viscosity, lubrication properties, solubility and solvation power, boiling points and melting points. However, since current GC-MS can not be properly used for isomer distribution analysis in view of weakness or absence of there molecular ions, this important subject is being ignored and represents an untapped opportunity. The presentation will demonstrate how the provision of enhanced molecular ions combined with extended range of compounds amenable for analysis and faster analysis dramatically improves GC-MS analysis of real petrochemical fluids and how it enables isomer distribution analysis for unique fuels and oils characterization.

Keywords: Fuels\Energy\Petrochemical, Gas Chromatography/Mass Spectrometry
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography/Mass Spectrometry
There is a considerable amount of research focused on the formation of bioethanol using nonfood biomass feedstocks (lignocellulose, hemicellulose and cellulose). Studies indicate that by 2015, bioethanol production will exceed the needs of E10 fuels, resulting in an oversupply of ethanol. Consequently, bioethanol appears to be the platform molecule for the production of value-added chemicals in the next decade. The ethanol-to-ethylene conversion using various zeolite catalysts is well documented. The role of the catalyst in ethanol stream reforming (ESR) continues to be of interest.

The catalytic conversion of ethanol appears to be an ideal process to use as a benchmark for system performance: ethanol is stable, and the ethylene selectivity is high using readily available zeolite catalysts. This work describes the findings of an evaluation of the Tandem Micro-Reactor GC/MS system based on the conversion of ethanol. The results of the following tests will be reported:
1. Monitor hydrocarbons formed as the temperature of the catalysis is heated at a fixed rate.
2. Monitor hydrocarbons formed at selected catalyst temperatures.
3. Determine the amount of sample converted to char using EGA-MS.
4. Monitor the surface activity of the catalyst using ethylene as a test probe.

Keywords: Biofuels, Fuels\Energy\Petrochemical, Gas Chromatography/Mass Spectrometry, Process Analytical C
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography/Mass Spectrometry
Electrochemical Techniques for Biomedical Monitoring

Electrochemical Analysis of Metabolic Flux in Nanoliter Samples from Organ-on-a-Chip Systems

Long term interconnected organ-on-a-chip systems (OoC) present the opportunity to more accurately predict patient reactions to pharmaceuticals than traditional cell culture protocols. Integrating heart, lung, liver, and kidney OoCs, presents an opportunity to observe how drug metabolism in the liver as well as the resulting side reactions could affect other organs in the system. To determine the long term effects of particular stimuli, effluent from the system should circulate through each of the organs, just as blood continually circulates through the human body. For meaningful information to be determined from studies of this nature, the health of the organs needs to be monitored without sacrificing excessive amounts of fluid. Previous work has shown the detection of glucose, lactate, oxygen and acid levels using modified screen printed electrodes in a 26µL chamber; however, measurements on smaller volumes are necessary to avoid altering the system significantly. A new platinum electrode array has been fabricated using electron beam deposition and soft lithography, and modified through electrodeposition and ink-jet printing techniques. Upon modification, the sensor array was capable of measuring glucose, lactate, oxygen and pH as well as significant changes in solution conductivity. Electrochemical detection occurs in a multichannel PDMS housing defining an approximately 350nL sample chamber.

This work was funded by the Defense Advanced Research Projects Agency (DARPA W911NF-12-2-0036) and Vanderbilt Institute of Chemical Biology.

Keywords: Array Detectors, Biosensors, Electrochemistry, Lab-on-a-Chip/Microfluidics
Application Code: Biomedical
Methodology Code: Electrochemistry
Abstract Text

Reactive oxygen species (ROS) play a vital role in the etiology of disease in vivo. Physiological levels of these species appear to be involved in myriad of physiological and pathological processes. Superoxide radical (SO), in particular, plays a significant role in ischemia-reperfusion injury in the brain. Real-time monitoring of superoxide dynamics in vivo would provide useful insight into the evolution of this free radical, but real-time monitoring has not been achieved due to the lack of adequate methods. This presentation will discuss the use of electrochemical, Cyt C microbiosensor that can reliably measure the temporal evolution of superoxide continuously in a rat brain in vivo. We will also discuss the dynamics of superoxide evolution during the progress of ischemia-reperfusion (I-R) injury in a rat model of stroke. Additionally, we studied the role of ceria nanoparticles as a model antioxidant and explored their capacity to scavenge the superoxide and to use them as a potential therapeutic target during I-R injury. We will present the ability of our biosensor to examine, in situ in intact animals, the scavenging ability of Cerium oxide nanoparticles and their contribution in controlling SO levels during normoxic, ischemic and reperfusion periods in whole animals.

Keywords: Biosensors, Electrochemistry, Microelectrode, Nanotechnology

Application Code: Biomedical

Methodology Code: Electrochemistry
Electrochemical Techniques for Biomedical Monitoring

Wireless Microfluidic-Based Biosensor Auto-Calibration System with Real-Time Concentration Display

Real-time monitoring of chemical changes in patients is currently an area of great interest, as it provides the possibility of real-time feedback of the patient’s condition to the clinical team. However, there are two main barriers to accomplish this:

1. Real-world sensors or biosensors do not maintain constant sensitivity and offset during long-term monitoring.
2. The clinical team need chemical concentrations from biosensors to have the physiological understanding of the patient’s condition.

Currently, most research monitoring devices give signal output of voltage, rather than concentration, hence they are not able to provide real-time clinical feedback to improve the treatment of patients, due to these two conflicts.

We have designed a novel miniaturised wireless microfluidic-based sensor auto-calibration system, with on-board high-precision data converter, high-speed micro-processor, and relevant user interface, which allows users to set up relevant calibration parameters seamlessly. After every calibration process, the processor will automatically calculate and store the calibration curve, and fill the most up-to-date sensitivity into the monitoring system. For each sensor channel, the system provides two different outputs: raw voltage signal, and processed real-time concentration. The controlling application software has been implemented on computers and mobile devices (e.g. smart phones and tablets).

The experimental data from different applications will be presented.

Keywords: Biosensors, Calibration, Electrochemistry, Lab-on-a-Chip/Microfluidics
Application Code: Biomedical
Methodology Code: Electrochemistry
Real-time in vivo measurements of neurotransmitters such as dopamine are critical for advancing research in biology, psychology and pharmacology. Fast scan cyclic voltammetry (FSCV) coupled with carbon-fiber microelectrodes is a proven technology for monitoring rapid chemical changes in the brain and associating these changes with behavior. Recent advances have enabled wireless system capable of making in vivo FSCV measurements, allowing animals more unrestricted motion and behavior. However, power consumption and data transfer requirements have significantly limited further development and application of these wireless systems. Traditionally, in vivo FSCV collects 10 CVs per second with 1000 data points per CV. In this work, the in vivo performance of FSCV was evaluated at reduced sampling rates and with reduced data points per CV. The research demonstrates that FSCV applied at as low as 1Hz with 100 data points per CV is still capable of detecting spontaneous dopamine fluctuations in freely moving animals. Moreover, the effects of cocaine on electrically-evoked dopamine kinetics and spontaneous dopamine dynamics in behaving animals was studied at reduced sampling rates. Utilizing the 1 Hz sampling rate at 100 data points reduces the quantity of data generated per second by two orders of magnitude compared to the traditional protocol, and is expected to decrease the power consumption by a similar value. This research will help guide development of wireless FSCV systems while retaining their performance for in vivo measurements.

This work was supported by the ASSIST Center (NSF NERC EEC-1160483) and NIH (NINDS NS076772).

Keywords: Biomedical, Detection, Electrochemistry, Monitoring
Application Code: Biomedical
Methodology Code: Electrochemistry
Novel methods of culturing including the use of bioreactors and organ on a chip environments and an increasingly diverse range of molecules of interest require new approaches to monitor the metabolic behavior of mammalian cell cultures. Platforms such as bioreactors can provide a more in vivo-like environment than traditional two dimensional platforms for cell cultures and as a result may dramatically affect the yield of biomolecules and the signaling molecule profile. Unfortunately, non-traditional approaches can complicate standard methods of metabolic monitoring such as optical spectroscopy. A screen-printed electrode array and accompanying fluidic control system has been developed for real-time monitoring metabolically significant molecules such as glucose, glutamate, lactate, and oxygen as well as observing changes in solution pH. Flexible methodologies have been developed to allow a variety of choices in the monitored analytes and applicability to diverse culturing platforms. Monitoring of up to four analytes simultaneously and in real time is demonstrated. The sensors are low-cost, have a lifetime of several weeks, and can be recycled for use in multiple experiments and for the detection of different analytes. The capabilities of this method have been demonstrated by the real-time monitoring of cellular responses to well-studied metabolic stimuli and validated by comparison to established methods for 2D cultures.

Keywords: Bioanalytical, Biosensors, Clinical/Toxicology, Electrochemistry
Application Code: Biomedical
Methodology Code: Electrochemistry
Amperometric sensing is a sensitive, compact, and inexpensive approach to quantitative detection of nitric oxide (NO) in physiological systems. The current gold standard for direct gas-phase detection of NO is chemiluminescence, which is both sensitive and selective, but remains impractical in a clinical setting due to its cost and footprint. While many amperometric sensors exist for the detection of NO as a dissolved gas in aqueous environments, direct electrochemical gas-phase detection for biomedical applications remains largely unexplored. We have adapted a solid-polymer electrolyte membrane-electrode preparation in the development of an amperometric NO sensor capable of direct gas-phase detection. Nafion, a cation exchanger, can be impregnated with Pt ions, which are then chemically reduced to a porous Pt metal in the presence of a reducing agent such as sodium borohydride. The resulting Pt/Nafion membrane-electrode can then be fashioned into a sensor with high sensitivity, low limits of detection (as low as 3.7±0.5 ppbv), fast response times (<10 s), and low cost. Potential applications of this sensor include the NO sensing of exhaled human breath and NO released from novel NO-donor-based biomaterials.

Keywords: Biomedical, Biosensors, Electrochemistry, Voltammetry
Application Code: Biomedical
Methodology Code: Other
Electrochemical Techniques for Biomedical Monitoring

Conductivity as a Sensing Tool for Monitoring Magnesium Corrosion Under Cell Culture Conditions

Because controlling the corrosion rate of magnesium metal will be crucial to the success of biomedical implants containing pure magnesium or magnesium alloys, many ways have been sought to improve in vitro tests to analyze corrosion rates, and also to identify new methods of preparing or post-processing magnesium. In this work, for an in vitro assay, we explored the use of a commercially available conductivity sensor alongside a commercially available assay for magnesium ions to characterize magnesium corrosion under cell culture conditions that duplicate many physiologically appropriate parameters. With this assay system, we studied the corrosion of two previously untested magnesium single crystal samples that differed in surface treatments that could alter corrosion rates.

Abstract Text

Keywords: Biomedical, Characterization, Electrochemistry, FTIR
Application Code: Biomedical
Methodology Code: Electrochemistry
Real-time monitoring of patient’s biochemical changes requires well characterised biosensors that are fast responding, with analytical performance that is quantified during the period of use in for example the operating room or intensive therapy unit. Additionally, there is a great need for miniaturisation of such devices in order to reduce analyse consumption and critically reduce the instrument footprint in such congested hospital areas. Real-time measurement of the biochemical changes is most easily achieved using microdialysis as the sampling probes are FDA approved, but such probes typically produce dialysate for on-line analysis at 10-30 nl/sec. We have perused the development of microfluidic ‘circuits’ using LabSmith component that can give us precise fluid delivery, excellent temporal control of multiple liquid streams. Custom electronic controlled by ‘Apps’ provide a complete solution for new biosensor development and use. New sensors can be optimized, calibrated automatically and on-board signal processing allows a direct concentration output. Examples will be shown for a range of new sensors including novel carbon based electrodes.
We report a unique colorimetric sensor array based on a single ionic liquid (IL) for accurate detection and identification of closely related organic solvents and mixtures. This unique array was fabricated by ion-pairing bromothymol blue (BTB) anion with trihexyl(tetradecyl)phosphonium ([P[6614][sub]2][sub][BTB]) cation. Based on the concentration dependent response of this IL, four concentrations of [P[6614][sub][2][sub][BTB]] were used as individual sensors to fabricate this sensor array. Eight alcohols and seven binary mixtures of ethanol and methanol were examined to provide a stringent test for the proposed sensor array. Reproducible UV-visible absorption peak ratios were obtained for each sensor. These peak ratios were used to develop predictive models for alcohols and mixtures identification. We demonstrate that this array can identify eight alcohols and mixtures with 96.4% to 100% accuracy. Overall, this array is very promising for facile, inexpensive, rapid discrimination of structurally very similar solvents and solvent mixtures.
In this study, a simple, label-free, sensitive and selective DNAzyme-based QCM-D sensor for rapid detection of toxic metals was developed. A quartz crystal microbalance with dissipation monitoring (QCM-D) biosensor was used for highly sensitive and specific detection of toxic metal ions. Oligonucleotide-functionalized gold nanoparticles were used for both frequency and dissipation amplification to enhance the detection sensitivity. Metal-specific DNAzymes were immobilized onto the QCM-D sensor surface and allowed to hybridize with substrate-functionalized AuNPs. The DNAzyme catalyzed substrate cleavage in the presence of metal ions, causing the cleaved substrate-functionalized AuNPs to be removed from the sensor surface. Metal ions were determined by monitoring the change in frequency and dissipation signals. Detection limits in the nanomolar range for both frequency and dissipation measurements were obtained. The sensor showed excellent selectivity toward the targeted metal ions in the presence of other metal ions. The proposed sensor system described here represents a new class of metal ion sensor, and its simple detection strategy makes it possible for pollution-free detection and could find applications in the on-line water quality monitoring.
Harmful algal bloom (HAB) events have become more prevalent in the last few decades, with each event leading to the potential release of biotoxins in our waterways. To prevent detrimental damage to the environment and human health, bloom monitoring via analytical methods is essential. Typically this is done by direct cell counting using light microscopy with species identification based on cellular morphology. This method is extremely time consuming and is subject to false identification as some toxic and non-toxic species share similar morphologies. More recently, it has been shown that there is a variable rRNA region in HAB species that can be targeted with complimentary, specific oligonucleotide capture probes.

Here, we demonstrate the covalent attachment of amine modified oligonucleotides to aminated surfaces using a thiocyanate crosslinker. Thiolated oligonucleotide attachment to vinyl surfaces using thiol-ene click chemistry methods has also been shown. In both methods, fluorescently labelled oligonucleotide target sequences have been used to identify and optimise oligonucleotide attachment via a model system. This chemistry is being integrated with nanoparticle surfaces to determine if an increase in oligonucleotide capture probe density per unit area is able to increase overall detection sensitivity. The alternative surfaces will be compared and discussed with future work looking to apply the modified surfaces to cultured and natural lysed cell samples.
Mycotoxins are toxic secondary metabolites produced by fungi, which can exist in food as a result of fungal infection of crops. Their strong resistance to decomposition and digestion cause mycotoxins to remain in the food chain. The analysis of mycotoxins in food and animal feed has been a challenge mainly due to the complexity of food matrices and desired low detection limits. In recent years, significant advances in the analytical techniques were applied to the detection of mycotoxins. There has been an increasing need for a method to detect multiple mycotoxins with a single sample preparation and analysis method.

Previously, research concentrated on an LC-MS/MS method for multi-mycotoxin analysis as mass spectrometry provides appropriate selectivity and sensitivity for detection. This study investigated the selectivity for over 15 common mycotoxins on a variety of solid-core and fully porous sub 2µm HPLC columns with different stationary phase chemistries. The results of these analyses were evaluated for optimum resolution and selectivity, and the conclusions will be presented.

Keywords: Food Contaminants, Food Safety, Liquid Chromatography/Mass Spectroscopy
Application Code: Food Safety
Methodology Code: Liquid Chromatography/Mass Spectrometry
### Session # 1760  Abstract # 1760-2

**Session Title**: Food Safety (Half Session)

**Abstract Title**: **What Can You Do with a $40 Do-It-Yourself Spectrometer?**

**Primary Author**: Yagiz Sutcu  
**Author**: InfoScope Research

**Co-Author(s)**: Aysegul Ergin

**Date**: Wednesday, March 11, 2015 - Morn  
**Time**: 08:50 AM  
**Room**: 267

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**Abstract Text**

Spectroscopic techniques such as ultraviolet-visible (UV-Vis) and near-infrared (NIR) spectroscopy are common analytical tools used in many areas including environmental monitoring, food safety and quality, pharmaceuticals, bio-imaging etc. Fortunately, spectroscopic instruments are now available in handheld/portable form and do not require specialized laboratory facilities to operate. These portable devices are easy to use with minimum user training, and once the spectral signatures are developed and validated, suspected products can be tested within a few seconds. With all these advantages and the availability of cloud-like platforms, portable spectroscopic instruments can easily be used in many places. Furthermore, due to the availability of very low-cost HD-webcams and 3D printing, do-it-yourself (DIY) spectrometers become much more accurate, reliable and accessible.

In this study, we investigated the capabilities and limitations of portable, extremely affordable, webcam-based UV-Vis DIY spectrometer in the food safety and quality applications. More specifically, we focused on edible oil adulteration detection and added sugar detection in fruit juices. Our preliminary results showed that despite its simple design and low-cost, this webcam-based UV-Vis DIY spectrometer is a very promising tool which can be used by consumers to fight against food fraud. Furthermore, our results indicate that similar approach can be used to detect not only other types of food fraud and adulteration (milk, fruit juices, honey etc.) but also to identify oil contamination in water and soil. However, it is crucial to collect/maintain a nice library of spectral data and efficient and scalable algorithm in order to be able to achieve acceptable accuracy.

**Keywords**: Food Identification, Food Safety, Spectrometer, UV-VIS Absorbance/Luminescence

**Application Code**: Food Safety

**Methodology Code**: UV/VIS
**Abstract Text**

Although the European Food Safety Agency has not yet established the migration level of many chemicals in foodstuffs, several classes of contaminants are commonly found in food packaging prepared from recovered paper fibers. Rapid and reliable analytical methods are needed for identifying and quantifying target contaminants in recycled paper and board in order to prevent food contamination. In the present work, a method suited to in-process quality control of recycled board for food contact applications is presented developed using benzophenones, phthalates and diisopropyl naphthalene isomers as contamination markers of recycled paper and board materials. The methods involve a sample pre-treatment by Accelerated Solvent Extraction, evaporation and SPE purification, followed by analysis with GC-MS.

Results will be shown of the analyses of commercial recycled board to validate method’s applicability. Due to its characteristics of rapidity (less than 1 hour per sample), good sensitivity and partial automation, the proposed method may help food packaging manufacturers to keep contamination levels of recycled paper and board under control. Furthermore, a comparison of the performances achieved for the proposed method using Ion Trap MS and GC-Single Quadrupole MS will be presented.

**Keywords:** Accelerated Solvent Extraction, Food Contaminants, GC-MS, Paper/Pulp

**Application Code:** Food Safety

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Aminoglycosides are a well-known class of antibiotics that are routinely monitored in animal-derived foods. Many countries have instituted regulatory limits for aminoglycosides. There is growing concern of the impact that these microbial resistant compounds have on human health and development. A sensitive and robust analytical method is required in order to enforce the regulations and ensure the safety and quality of the food supply.

This study compared the USDA sample preparation method for aminoglycosides in porcine tissue with a new method utilizing molecularly imprinted polymer (MIP) solid phase extraction (SPE). Older methods for sample preparation of aminoglycosides have used cation exchange SPE. The recent technology of molecularly imprinted polymer SPE can provide improved selectivity toward analytes and better removal of non-target matrix components. The MIP SPE procedure has the potential to offer improved cleanup as well as reduced sample preparation time.

Both cation exchange SPE and MIP SPE procedures were applied to the porcine tissue sample spiked with ten aminoglycosides. The samples were analyzed by LC-MS/MS using matrix-matched calibration curves. The results of this comparison will be presented and the overall method performance of two different sample preparation methods will be discussed.

**Keywords:**  
Food Safety, Liquid Chromatography/Mass Spectroscopy, Solid Phase Extraction

**Application Code:**  
Food Safety

**Methodology Code:**  
Sampling and Sample Preparation
The application of electrospun nanofibrous substrates for surface-assisted laser desorption/ionization (SALDI) mass spectrometry was evaluated. SALDI substrates can yield high quality mass spectra without the use of a matrix, which can often hinder analysis through signal interference or formation of “sweet spots”. Electrospinning is a fast and simple technique that can easily generate these substrates by applying an electric potential between a polymer solution and collector plate. These nanofibrous substrates can have a range of diameters and morphologies, and typically have very high surface area to volume ratios. This makes them ideal candidates for SALDI analyses. Polymeric nanofibers with different functionalities were prepared from commercially available, low-cost polymers: polyacrylonitrile (PAN), poly(vinyl alcohol), (PVA), and an epoxide-based photoresist (SU-8). These electrospun polymers were used as substrates in the analysis of peptide and protein standards, covering a wide range of molecular weights. Improved ionization efficiency and signal to noise (S/N) ratios was observed, particularly for high molecular weights compounds outside of the normal range for SALDI (>150,000 Da). The SALDI results also show enhanced shot-to-shot reproducibility compared to matrix-assisted laser desorption/ionization (MALDI). Further improvements in signal-to-noise ratios are obtained using matrix-enhanced surface-assisted laser desorption/ionization (ME-SALDI) compared to either MALDI or SALDI methods.

This research was funded by the National Science Foundation.

**Keywords:** Mass Spectrometry, Nanotechnology, Peptides, Protein

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
Laser Ablation/Desorption/Nanopeptides Applications of Mass Spectrometry

Wavelength Dependence of Tip Enhanced Laser Ablation for Mass Spectrometry

We have recently developed a method for laser ablation of materials at micrometer size scales using the conducting tip of an atomic force microscope (AFM) to direct the ablation to a small size. Initial studies were performed with a 355 nm nanosecond pulsed Nd:YAG laser, which was able to ablate peptides and proteins with minimal fragmentation from micrometer or smaller spots. In this study, we have expanded the wavelengths tested from the UV to the visible and infrared wavelengths. The Nd:YAG laser provides output at wavelengths of 532 nm in the visible and 1064 nm in the near IR in addition to the 355 and 266 nm UV wavelengths. A wavelength tunable mid-IR optical parametric oscillator operating in the 3 µm wavelength region has also been tested for tip-enhanced ablation. In this work, an AFM is used to position a 30 nm radius gold-coated silicon tip at a distance of approximately 10 nm from the sample surface for material removal. The laser is mildly focused onto the target and the fluence is set to just below the far-field threshold to irradiate the AFM tip for material removal with a smaller spot size than a laser focused with a conventional lens system. The AFM is used to image ablation craters and place the tip at the area being analyzed. The captured material is deposited on a MALDI target or electrosprayed for mass spectrometry analysis. The effect of wavelength on spot size, transfer efficiency, and fragmentation will be reported.

Keywords: Laser, Laser Desorption, Mass Spectrometry, Small Samples
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Nanoelectrospray ionization (nanoESI) is a soft ionization technique for mass spectrometry that is widely applied to determine the molecular weight of biological macromolecules. Presently, common ESI emitters possess orifice diameters of 50-100 µm, and nanoESI emitters have diameters as low as 1-2 µm. Work presented here demonstrates the ability to perform nanoESI from capillaries pulled to orifice diameters less than 100 nanometers and discusses the fundamentals of nanoESI from these small emitters. Smaller nanospray orifice dimensions are shown to increase charge/droplet ratios, and thereby improve ionization efficiencies, and dramatic shifts in average charge state have been observed with these small orifice emitters. Here, important characteristics, such as flow rate, spray current and analyte influence, relevant to ESI-MS are examined for nanopipette emitters. Our results demonstrate that use of nanopipettes as nanospray ionization emitters is a promising new approach for ultra-trace biological mass spectrometry.

Keywords: Electrospray, Mass Spectrometry, Sample Introduction, Small Samples
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Measurement of glucosidase activity is of significance to diagnosis of certain diseases such as Gaucher disease. We report here a method to determine glucosidase (GD) activity using matrix-assisted laser desorption/ionization (MALDI)-time-of-flight (TOF) mass spectrometry in combination with novel surface chemistry. A new carbohydrate probe has been synthesized for capture of the reduced species and the probe is fixed to a fluorinated surface to generate a microarray. To measure the activity of alpha-GD, maltose has been used as a substrate. After reaction and washing with water, the noncovalent array was characterized by MALDI-TOF/MS. By measuring the ratio of intensities from peaks of the substrates and the products, we can accurately determine the level of enzyme activity. Using this method, we have also determined the half maximal inhibitory concentration (IC50) of acarbose and epigallocatechin gallate, and the values agree well with the reported IC50. Our approach is advantageous as compared to conventional colorimetric method that suffers from matrix interference in samples by removing matrices using the polyfluorinated surface.
We describe pressure-driven manipulation of nanoliter volumes of fluids with nanopipettes for fluid aspiration and dispensing. The fundamental behavior of fluids confined in the narrow channels of the nanopipette shank was studied to optimize sampling volume and probe geometry. Matrix Assisted Laser Desorption/Ionization mass spectrometry was utilized to characterize nanoliter volumes (<10 nL) of analyte sampled from \textit{Drosophila melanogaster} first instar larvae and single \textit{Allium cepa} cells with nanopipettes of diameter ~600 nm. Nanopipettes are thus shown to be a versatile tool that might find further application in studies of sample heterogeneity and population analysis for a wide range of samples.

**Keywords:** Bioanalytical, Mass Spectrometry, Sampling, Small Samples

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
**Abstract Text**

Lipids are important inherent components in cell membranes, which play significant roles in cell function. Imaging mass spectrometry methods including secondary ion mass spectrometry (SIMS) and matrix-assisted laser desorption ionization (MALDI) are promising techniques to identify and map different lipids in tissues and cells. SIMS and MALDI can provide complementary information. SIMS provides detection of small molecules at high spatial resolution while MALDI is capable of ionizing larger molecules of the sample albeit at reduced spatial resolution. Combining the data obtained from the two MS approaches can maximize the information available in biological analyses. Although these MS methods have associated abilities there are some limitations with each technique. In MALDI imaging, we replaced deposition of the organic matrix with a spray-on procedure for Au nanoparticle deposition to enhance the spatial resolution possibilities. This allows lipid molecular ion species and the relevant mass fragments to be imaged with enhanced spatial resolution. Additionally, we have combined this with backside laser illumination providing a high focused laser beam (~1 micron) and further enhancing spatial resolution. In SIMS imaging, we have used gas cluster ion beams (GCIBs) that have been developed for analyzing and imaging organic compounds such as lipids and metabolites of biological samples in the molecular ion mass range. Here, we show that improvements of these MS techniques for imaging of different types of lipids creates a potentially overlapping cross point that can deliver complementary data for molecular lipid imaging in biological tissue sections.

**Keywords:** Imaging, Laser Desorption, Lipids, Mass Spectrometry

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
Collision Cross Sections for 20 Protonated Amino Acids: Comparison of FTICR-MS, IMS and TWIMS Results

CRAFTI (cross sectional areas by Fourier transform ion cyclotron resonance) is a novel technique for measuring molecular size that is based on measuring dephasing rates for the transient signal in FTICR-MS (Yang et al., Anal. Chem. 2012, 84, 4851). This study compares collision cross sections measured using CRAFTI with results from other experimental (ion mobility spectrometry, IMS and traveling wave ion mobility spectrometry, TWIMS) and computational methods.

Cross sections for 20 singly-protonated amino acid ions obtained using CRAFTI on a 4.7 T Bruker Daltonics APEX 47e FTICR were found to correlate well with the IMS measurements; the correlation between CRAFTI and IMS is roughly as good as the correlation between IMS measurements from different laboratories. CRAFTI measurements also correlate well with molecular surface areas calculated from low-energy structures predicted from molecular mechanics conformational searches, either by using CPK molecular model surface areas or by using MOBCAL trajectory method calculations (Shvartsburg et al. J. Phys. Chem. A 1997, 101, 1684). Further, CRAFTI cross sections exhibited the same structural trends as the other methods. For example, amino acids with aliphatic side chains have larger than average cross sections, those containing sulfur are smaller than average, and those that can internally hydrogen bond fold more compactly than those with less hydrogen bonding capability. CRAFTI thus shows great potential as an effective technique for experimentally measuring molecular conformations.

We gratefully acknowledge NSF (CHE-1412289) for its financial support.

Keywords: Amino Acids, Bioanalytical, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Since the early 1990s, hydrophilic interaction (HILIC) has been the most widely applied retention mode in liquid chromatography in the pharmaceutical, biological, and food industries fields for the analyses of polar and/or ionizable compounds. It is commonly accepted that the retention of these compounds is controlled by both the adsorption onto the adsorbent and by the partitioning mechanism into the diffuse water-rich layer that is formed when the HILIC material is in contact with acetonitrile-rich eluents.

An unsolved enigma in HILIC is to assess the relative contributions of adsorption and partitioning retention mechanism to the overall, observed retention factor. The mere measurement of the sole retention factor is not sufficient because it only provides the sum of these two separate contributions. Therefore, the challenge is to propose and validate an original approach that can unambiguously decouple the adsorption from the partition process of small, polar and ionizable compounds in HILIC.

In this presentation, we discuss a method that consists in measuring both the retention factor and the intra-particle diffusivity. This latter property is crucial in HILIC because adsorbed molecules (zero mobility) and those concentrated in the diffuse water-rich layer (finite mobility) participate differently to the overall intra-particle diffusivity. This method is based on the validation of an original model of effective diffusion in porous particles in which the rigid adsorbed water, the diffuse water-rich layer, and the bulk eluent are considered as three different phases in equilibrium. This approach is applied to the determination of the HILIC retention mechanism of cytosine, nortriptyline, and nicotinic acid on a 3.5 [micro]m Bridge Ethyl Hybrid (BEH) and silica-diol adsorbents in contact with a series of ternary mobile phases ACN/water (10 mM acetate buffer pH=5)/third eluent (water, EtOH, THF, ACN, or n-hexane), 95/5/5, v/v/v.

Keywords: Adsorption, HPLC, Liquid Chromatography, Separation Sciences
Application Code: Drug Discovery
Methodology Code: Liquid Chromatography
Centrifugal Chromatography instrumentation is used in the natural products, pharmaceutical, biological, and in academia as a means of fractionating complex mixtures of organic components. Fast centrifugal partitioning chromatography [FCPC] offers several advantages versus traditional methods such as HPLC in that no solid packing material is required, which in itself can cross contaminate fractions. The FCPC is a discrete stage-wise device that uses the specific partitioning coefficients of the individual components for isolation of the product fractions. Due to the large quantity of extraction stages in the rotating column, components with similar molecular structures can be easily isolated. Recent improvements in the stage cell design, such as diamond shaped profiles versus traditional Z-cell profiles improve diffusion and reduce mobile phase holdup within the cells. Twin cell designs have been developed that improve resolution and degree of fractionation. FCPC technology is commonly used in isolation of botanicals such as Cannabis, Tobacco, Opiate Derivatives, and Nutraceuticals. Capacities range from the milligram scale for analytical scale quantities, up to preparative scale quantities in the gram scale, and production scale capacities are available for kilogram scale fraction collection. Developments have been made in the sample collection system software. Manual systems are available and they can be used in conjunction with a PLC. Commonly used solvent systems include the Arizona range, HeMWat, aqueous biphasics, and both polar and non-polar solvent pairs.

Keywords: Capillary LC, Chromatography, Liquid Chromatography
Application Code: Other
Methodology Code: Liquid Chromatography
Intermolecular interaction between solute molecules (2-phenoxypropionic acid) and functional ligands (-bromo-
cyclodextrin) on the stationary phase surface in a chiral HPLC system was studied by means of the moment analysis
method. At first, pulse response and peak parking experiments were carried out for measuring some parameters
concerning the column geometry, the adsorption equilibrium, and the mass transfer kinetics. Then, the values of the first
absolute moment ($I$) and the second central moment ($\mu'$) of elution peaks were analyzed by the moment equations,
which had been developed on the assumption that the reaction kinetics between the solute molecules and the functional
ligands was represented by the Langmuir type rate equation. Consequently, the flow rate dependence of HETP calculated
from $I$ and $\mu'$ was analyzed on the basis of the values of the parameters described above to derive the kinetic
information about the intermolecular interaction, i.e., the association rate constant and the dissociation rate constant. In
addition, the values of the association equilibrium constant were also obtained from $I$ as the related equilibrium
parameter. It was demonstrated that the combination of chromatographic experiments and the moment analysis was one
of effective strategies for the quantitative study on intermolecular interactions.

Keywords: Chiral Separations, HPLC, Liquid Chromatography
Application Code: Other
Methodology Code: Liquid Chromatography
In the manufacturing of Antibody Drug Conjugates (ADCs), small molecule linkers are used for connecting the cytotoxic agents to antibodies. The quality of the linkers is tightly monitored by a panel of analytical tests. The impurities present in these materials can be residual solvents, elemental impurities, moisture, degradation products, reaction byproducts and, if the molecule contains chiral centers, chiral impurities. Most common impurity assays on a release panel are GC- and HPLC-based assays that do not separate chiral isomers. Chiral purity is often controlled by the synthetic route itself and not directly assessed at the release. With recent advances in chiral stationary phases and increased availability and diversity of commercial chiral columns, development of chiral HPLC assays without derivatization has become more accessible. The development and evaluation of chiral methods for three linkers frequently used in ADCs will be presented. The different challenges faced during the assessment of racemic mixtures versus pure isomer product will be discussed. Linker 1 and 2 are examples of racemic mixture while linker 3 is a single isomer.
Carbon dots (C-dots) have attracted interest over the past few years because of their distinctive properties such as high photostability, chemical inertness, low cytotoxicity, and excellent biocompatibility when compared to metal-based quantum dots. These unique nanoparticles are being explored for a wide range of applications including bioimaging, optoelectronic devices, and metal-ions sensing, among others. C-dots are usually separated by low-resolution methods such as dialysis, centrifugation, and filtration. It has been showed that after these procedures they can still be a complex mixture. Individual components of this mixture can have different size, charges, and photoluminescence properties. In this work, we separated the C-dots mixture by means of size exclusion chromatography to obtain fundamental insight on its complexity. Some of the chromatographic peaks were collected for further characterization using Fourier transform infrared spectroscopy and transmission electron microscopy. This presentation will focus on the chromatographic behavior of C-dots samples on SEC columns under various chromatographic conditions.

**Keywords:** Chromatography, HPLC, Luminescence, Separation Sciences

**Application Code:** Nanotechnology

**Methodology Code:** Liquid Chromatography
Hydrophilic Interaction Liquid Chromatography (HILIC) is one of the fastest growing chromatography modes for separating polar and hydrophilic molecules. Most of the molecules of biological interest bear hydrophilic functional groups. The need to develop hydrolytically stable HILIC with novel selectivity for biological molecules cannot be overemphasized. Most of the HPLC stationary phases employ silica as a support because of its high efficiency and ease of chemical modification. However, attaching hydrophilic ligands on the silica for HILIC purposes comes with a challenge – such phases are hydrolytically unstable. Hydrolytic cleavage of the attached functional group and silica solubility in aqueous phases are the Achilles' heel in the column manufacturing process.

Our efforts are directed towards developing hydrolytically stable HILIC phases. This talk will discuss the trends and approaches in increasing the hydrolytic stability of HILIC phases using simple synthetic routes and novel silane designs. Using multipoint attachment technology for cyclofructan based columns, novel dipodal silanes functionalized with zwitterions, and employing base stable high efficiency silica, one can synthesize a series of hydrolytically stable HILIC with desired unique selectivity.

Keywords: Chiral, HPLC Columns, Liquid Chromatography, Modified Silica

Application Code: Materials Science
Methodology Code: Liquid Chromatography
We have applied a two-step procedure for the identification of the \( \text{C}_{26}\text{H}_{16} \) polycyclic aromatic hydrocarbons, PAHs, present in a sample of coal tar. First, we have used normal-phase liquid chromatography for the fractionation of the coal tar products to isolate the fractions with the \( \text{C}_{26}\text{H}_{16} \) PAHs, and second, we have applied a combination of liquid chromatography with ultraviolet-visible spectroscopy (LC/UV-Vis) and gas chromatography with mass spectrometry (GC/MS) with the purpose of establishing the unequivocal identity of the \( \text{C}_{26}\text{H}_{16} \) PAHs. As an essential part of the procedure, we have developed suitable chromatographic methodologies that allow the separation of 17 \( \text{C}_{26}\text{H}_{16} \) PAHs by LC/UV-Vis and GC/MS. These methodologies included the investigation of the elution behavior and the selectivity of two commercially available LC columns and two commercially available GC columns. In addition to the UV-Vis spectral information from reference standards and from literature references, the positions of the UV-Vis spectral bands of unknown PAHs have been theoretically predicted by means of the Annellation Theory.

Keywords: Coal, GC-MS, HPLC, UV-VIS Absorbance/Luminescence
Application Code: Environmental
Methodology Code: Liquid Chromatography
Abstract Text

The Chemical Weapons Convention prohibits the development, production, acquisition, stockpiling, retention, transfer or use of chemical weapons by Member States. Verification of compliance or investigations into allegations of use requires accurate detection of chemical warfare agents (CWAs) and their degradation products. Detection of CWAs such as organophosphorus nerve agents in the environment relies mainly upon the analysis of soil. We now present a method for the detection of the nerve agent VX and its hydrolysis products by analysis of ethanol extracts of contaminated white mustard plants (Sinapis alba) which retained the compounds of interest for up to 45 days, providing a simpler sampling strategy, and protection against environmental loss of the nerve agent.

Sinapis alba was grown in VX, MPA and EMPA spiked (250 µg ml-1) clay, loam or sandy soil. The different soils moderated the rate of initial uptake of VX but not the longer term uptake: the duration the VX was detectable (to sub 1 ng of VX per plant) in the plant was the same regardless of the soil type. In addition, the plants metabolised the VX to EMPA and MPA and interestingly the MPA found in the plants increased when they were grown in VX-spiked clay. VX, EMPA and MPA were detected in the plants for up to 45 days. The data suggest that the EMPA found in the plants grown in soil contaminated with VX originated from metabolism of VX demonstrating a possible use for bioremediation of contaminated land.

The method described for detecting nerve agent residues in the environment will help the OPCW and CWC Member States confirm their presence in future. The use of localized samples and a simple extraction procedure will increase the probability of discovering nerve agent use. The ability of plants to absorb nerve agents suggests that green manures might be useful for remediating nerve agent-polluted sites.

Keywords: Environmental, Forensics, Gas Chromatography/Mass Spectrometry, Liquid Chromatography/Mass S
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
Matrix Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-MS) is an analytical technique used most often for thermally unstable large molecules, like proteins and polymers. The matrix is used to transfer laser energy and aid in desorbing and ionizing the sample of interest. Unfortunately, as low mass species, the matrices often interfere with spectra in the low mass range. Their peaks are larger than and in the same area as sample peaks leading to the need to find alternative assisting mediums.

The aim of this project is to use nanoparticles to assist in the desorption and ionization of low mass forensically relevant samples, not normally analysed via LDI-MS. This research is significant due to the fact that the MALDI, as an instrument, has various advantageous qualities including soft ionization and sensitivity, as well as rapid analysis via Mass Spectrometry. This could result in samples being analysed and identified with relatively minimal sample preparation.

Samples were analysed either in solution or from laden fibres of paper adhered to tapes of varying conductivity. They were then treated with a small amount of either silicon or gold nanoparticles. The MALDI was set to low mass range (less than 400 Daltons) in positive reflectron mode.

This project identifies the potential of a technique traditionally used for the analysis of biological and high mass samples, opening it up to uses in the forensic field. This is done by making use of new and popular innovative materials, nanoparticles.

Funding for this project is provided by Flinders University.

Keywords: Forensic Chemistry, Laser Desorption, Nanotechnology, Time of Flight MS
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) (Smith & Spanel, 2005) is a real-time analytical technique that offers potential for rapid screening of volatile organic compounds (VOCs) to ultra-trace levels in air (Prince et al., 2010). Quantitation limits in the low part-per-trillion range (by volume; pptv) are achieved without sample preparation or preconcentration and results compare well with those obtained at an accredited laboratory using the United States’ Environmental Protection Agency (US EPA) TO-15 Compendium Method (Langford et al., 2014).

This paper presents the results obtained during development of a rapid analytical method that targets the compounds in the US EPA TO-14A Compendium Method. A Syft Technologies Voice200Ultra SIFT-MS instrument was used in this study. The quantitation limits specified by the TO-14A method are achieved within a few seconds for each analyte. SIFT-MS also offers significantly enhanced dynamic and linearity ranges compared to the canister-GC/MS method.

Direct mass spectrometry techniques, such as SIFT-MS, cannot resolve all isomers as required by the TO-14A method. However, this study has demonstrated that SIFT-MS provides a rapid screening tool that saves busy laboratories significant money through pre-screening of incoming samples prior to regulatory analysis. These results also form the basis of deployment of SIFT-MS instrumentation as real-time analyzers for TO-14A method compounds, such as in ambient or fence-line monitoring scenarios.

Acknowledgement: This work was funded by Syft Technologies Ltd, New Zealand.


Keywords: Environmental/Air, Mass Spectrometry, Ultratrace Analysis, Volatile Organic Compounds
Application Code: Environmental
Methodology Code: Mass Spectrometry
Cold vapor generation (CVG) is an alternative approach for determination cadmium (Cd) by atomic and mass spectrometry techniques. Extensive research has been done so far, but the performances of the analytical methods reported for Cd vapor generation has been far from being optimum in comparison to those of other hydride and vapor forming elements, such as arsenic, selenium and mercury. In this work, Ti(III) and Ti(IV) were utilized as a catalytic additive to develop highly efficient vapor generation methods for determination of Cd by CVG-ICPMS in complex samples. Acidified sample solutions were mixed online with KCN and Ti(III) or Ti(IV) solutions and then reacted with NaBH4. Vapor generation conditions for Ti(III) and Ti(IV) were examined using HCl and HNO₃ within a range from 0 to 12% (v/v). Ti(III) provided optimum conditions in HCl-KCN medium, while highest efficiency was obtained in HNO₃-KCN medium with Ti(IV). On-line mixing sequences were varied using different manifold settings to elucidate the roles of Ti solutions. The results indicated that presence of Ti(III) or Ti(IV) was essential regardless of the mixing sequence. Sensitivity was improved about 50-fold. A detection limit of 3 ppt and 6 ppt were obtained for HCl-KCN-Ti(III) and HNO₃-KCN-Ti(IV) system, respectively. No significant interferences were noted from transition metals at 1.0 ppm levels. Among hydride forming elements, Bi, Sb, Sn and Pb induced suppression above 0.5 ppm levels. The procedures were verified by analysis different materials, including seawater, domestic sludge, liver and bone ash, for Cd by CVG-ICPMS.

Keywords: Elemental Mass Spec, Environmental Analysis, ICP-MS, Method Development
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The ambient desorption/ionization (ADI) sources for mass spectrometry have been in continuous evolution over the past decade. ADI sources produce ions via a ‘soft’ mechanism, the ionization of the sample occurs in an open ambient environment which leads to more efficient sample handling (i.e. high tolerance of sample size/shape/physical state). Marcus and co-workers have developed a liquid sampling-atmospheric pressure glow discharge (LS-APGD) as a low power, small footprint, and cost efficient ionization source for mass spectrometry. A glow discharge microplasma is sustained between the surface of an electrolytic solution, introduced through a 0.28 mm (i.d.) capillary housed inside a 1 mm (i.d.) metal capillary, and a stainless steel counter electrode mounted ~2 mm away, while helium sheath gas flows between the two capillaries. Previously, the LS-APGD was utilized as an elemental ionization source for direct liquid sampling with LODs ranging from tens to hundreds µg L⁻¹. The LS-APGD can be easily modified into an ADI source, utilizing the microplasma to efficiently desorb/ionize the sample directly from a surface when it is placed ~2-3 mm below the plasma.

Presented here, the ADI-LS-APGD is mounted to the existing electrospray ionization (ESI) interface of a Thermo Scientific LCQ Fleet Ion Trap Mass Spectrometer. The parameters investigated for optimization include powering mode, liquid flow rate, He sheath gas flow rate, plasma discharge current, and the sampling geometry (incidence angle and take-off angle) and sampling distance. A well characterized small molecule, caffeine was used as the test analyte. 100 µg mL⁻¹ caffeine in 50:50 MeOH/H₂O, was spotted on a glass slide, and oven dried. Intensity of the molecular peak (m/z=195), intensity of the major fragment (m/z=138), and signal-to-background ratio were factors for the optimization.
On-line mass spectrometry (MS) was implemented and developed as a tool to monitor and quantify ethanol vapor productivity from multiple laboratory photobioreactors. Oxygen generation and carbon dioxide utilization profiles were measured for increased process understanding and optimization. A magnetic sector MS was found to provide enhanced stability and accuracy relative to a quadrupole mass spectrometer. A modified ion source with glass lined entrance was utilized to enhance ethanol response and reduce detector settling time. Ethanol mass balance values, comparing on-line vapor MS measurements to liquid gas chromatography samples, were within 7%. Measured ethanol vapor concentration correlated well with values predicted by an ethanol-water vapor-liquid equilibrium (VLE) model (Aspen Plus). The MS technique is relatively simple to interface to equipment, requires no direct sample contact, requires minimal maintenance, allows sampling of multiple components simultaneously, and provides fast measurement for increased sampling frequency from multiple reactors. Use of MS significantly reduced process development time for effective strain selection and ethanol productivity optimization.

**Abstract Text**

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**Keywords:** Bioanalytical, Biospectroscopy, Biotechnology, Mass Spectrometry

**Application Code:** Fuels, Energy and Petrochemical

**Methodology Code:** Mass Spectrometry
Combustion and pyrolysis of solid fuels are extremely complex chemical processes, that are by far not fully understood. The gas phase chemistry of solid fuel combustion in the fuel’s voids and pores is difficult to address by conventional analytical methods. Recently photo ionisation mass spectrometry (PIMS) was established as a fast on-line analytical technique to analyse the chemical signature of highly dynamic combustion and pyrolysis processes in cigarettes during puffing. A new combination of PIMS and a capillary microprobe sampling system (μ-probe) was developed, allowing direct examination of the composition of organic vapours even in the centre of the cigarette’s combustion zone. This μ-probe-PIMS approach was further developed to a spatial and temporal resolved mapping method. Repetitive smoking experiments using a smoke machine and reference cigarettes were done. Different sampling positions in the cigarette tip were multiply addressed by μ-probe PIMS. The time resolved PIMS sequences were later combined to spatially resolved, time-dependent “maps” for the different compounds. This new technique for dynamic mapping was used to measure quantitative distributions of nitrogen monoxide, benzene and oxygen in the burning tip of a cigarette during a 2 second lasting puff (with 0.2s time resolution). The different formation and destruction zones of the investigated compounds in the reaction region and their dynamic changes were observed during the puff, and space-resolved kinetic data was obtained. For example, the classical formation and destruction mechanisms of NO during the puff (fuel-NO formation and re-burn in hydrocarbon rich zones) could be observed in a space- and time-resolved manner. The μ-probe-PIMS mapping technology can be applied for comprehensive chemical studies on various combustion or pyrolysis systems as well as on e.g. model systems for catalytic processes and thus is of large interest for fundamental and applied research.
A recent report from the Center for Disease Control and Prevention (CDC) has found evidence that current public swimming facilities may have inadequate methods for reducing human waste contamination. These methods include a variety of chemical treatments, filtration and required bacteria testing to ensure proper pool sanitation. One method employed to determine adequate sanitation is to measure E. coli concentrations. E. coli is a bacterium that is found in mammalian waste and surrounding runoffs into pools. Stercobilin is a mammalian metabolite found in both urine and feces. It has been demonstrated from previous studies that there is a positive correlation between E.Coli and stercobilin contamination in waste waters. Developing a method to quantify stercobilin concentration in public pools would allow for sensitive detection and a quicker result turnaround compared to current methods, thus allowing for facilities to quickly adjust water conditions. This presentation will focus on developing methods to extract a quantifiable amount of stercobilin utilizing solid phase extraction followed by analysis via Fourier Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometry. Discussion will also take place on developing a viable internal standard used for quantification of stercobilin within pool samples by isotopically labeling methods.
Autophagy is the main process for degradation of large cellular components and involves at least four different organelle types. Dysregulation of autophagy is a hallmark of cellular degeneration, including that observed in muscle degenerative disorders. Current monitoring of autophagy is limited to bulk measurements of autophagy markers such as LC3-II by western blot. These methods are inadequate to distinguish on what organelle type the bulk marker is found. A method based on individual organelle measurements would be ideal to distinguish marker levels on each organelle type. We previously reported analysis of individual autophagy organelles using GFP-LC3 as a marker. Unfortunately, this approach is inadequate for the analysis of autophagy organelles in tissue. This presentation will describe our efforts towards immunolabeling of autophagy organelles for analysis by capillary electrophoresis with laser induced fluorescent detection (CE-LIF).

C2C12 mouse myoblasts were chosen as proof of principle for antibody labeling. The post-nuclear fraction was labeled with DyLight-488 conjugated anti-LC3 antibody at concentrations ranging from 0-250nm. Samples were excited with an argon ion laser, fluorescence was spectrally resolved with a 560 dichroic long pass filter and selected with a 517.5-552.5 bandpass filter and detected by photomultiplier tube. Preliminary data from CE-LIF measurements shows that individual organelles appear as fluorescent events after 400s, indicative of their electrophoretic mobility. In addition, their fluorescent intensity is indicative of their LC3 levels. When no antibody was used, the fraction of false positives was 91.3%. On going experiments are the adaptation of this protocol to quantify LC3 levels in mouse skeletal muscle.

Keywords: Bioanalytical, Biological Samples, Capillary Electrophoresis, Fluorescence
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Magnetic beads represent a powerful analytical tool in purification methods, both as native silica particles and with adducted high-affinity molecules. We recently showed that DNA-paramagnetic silica particle interaction could be directly observed with the naked eye when exposed to a rotating magnetic field. This approach allows for quantification of DNA mass, based on the extent of bead aggregation, with femtogram sensitivity. Here, we exploit the inhibition of aggregation for the detection of DNA or RNA in a sequence-specific manner following isothermal amplification. The fragments of target sequences generated by loop-mediated isothermal amplification (LAMP) are too short to induce aggregation of the paramagnetic silica particles, however, they are effective at inhibiting aggregation in the presence of trigger DNA. Coupling LAMP with magnetic bead aggregation inhibition allows for the specific optical detection of bacterial and viral pathogens. We show the detection of various pathogens down to two copies in a manner that is comparable to conventional methods, but without the requirement for expensive instrumentation. More precisely, we show the strain-specific detection of Escherichia coli O157, a notable food-borne pathogen, as well as Rift Valley Fever Virus, a weaponizable RNA virus of national security concern. Finally, we show the potential for simple, optical, label-free real-time amplification capable of quantifying starting copy number in a manner comparable to traditional qPCR.
Quartz Crystal Microbalance (QCM) technology has proven to be an effective analytical tool for the real-time, dynamic, and quantitative analysis of mineral formation. One feature of the QCM that requires advancement for improved mineralization analysis is solution dynamics for controlled mineral precursor mixing. We present an analysis method that uses microfluidic laminar flow to achieve a liquid-liquid interface for controlled precursor reaction. This microfluidic device is integrated with QCM and optical microscopy to compare mass analysis with morphology of forming mineral. The new technique is much needed for the analysis of biomimetic mineralization templates, such as DNA aptamers.

Characterization of the instrument, in conditions of no mass loading, viscoelastic mass loading, and rigid mineral mass loading has been completed, demonstrating a widely applicable instrument. Analysis of unique mineralization templates shows distinct differences in heterogeneous and homogenous biomimetic template activity.

Keywords: Biosensors, Instrumentation, Lab-on-a-Chip/Microfluidics, Materials Science
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
While bacteria exhibit amazing diversity, the vast majority of species have yet to be studied by researchers, as retrieving cells from exotic environments and identifying the nutrients required to sustain their growth has proven exceedingly challenging. However, research suggests that collecting and attempting to cultivate a large amount of cells of an individual species significantly improves the chances that they will eventually be domesticated and grown in the laboratory.

We, therefore, have designed and fabricated polymer microfluidic devices that can enable the cultivation of microorganisms in their natural environment by targeting the differences in size between species. Previously, we showed that bacterial species in heterogeneous samples could be isolated with these devices. In this study, we integrate nano-porous membrane filters onto the devices to enhance the exchange of nutrients between large chambers in the device and the local environment. As cells respond to chemotactic signals and attempt to colonize empty chambers in the device, they become trapped in size-specific constrictions. Eventually, the cells grow and divide across the constrictions and populate the chambers with individual species.

We show that individual species can be isolated from liquid cultures containing multiple diverse microorganisms by simply immersing the devices into the sample and waiting. The devices were filled with deionized water, and a polycarbonate membrane filter, with 50 nm pores, was sealed over the access holes of the collection chambers, leaving the constrictions as the only point of entry. Our results demonstrate that these devices can be used in-situ to isolate microbes from the environment and potentially increase the discovery rate of new species.

This project was supported by NSF Award 1125535 and 1353853.
Microfluidics of Particles and Cells

Microfluidic Isolation and Enumeration of CD4+ T Lymphocytes from Blood Samples

The absolute number of CD4+ T lymphocytes in per microliter of adult blood plays a critical role in human immunodeficiency virus (HIV) infection implications and antiretroviral treatment (ART) assessment. A routine monitoring of CD4+ T lymphocytes every 3-6 months is recommended for patients of HIV infections at all stages. The most widely applied techniques in CD4+ T lymphocytes counting, such as multi-purpose and single-purpose flow cytometry, are limited to use in resource poor settings due to their high cost and complex operation. The alternative non-cytofluorographic methods, however, have limitations of low throughput, high labor requirements and lack of accuracy. Isolation of CD4+ T lymphocytes is complicated by interfering monocytes. An accurate, easily-operated, and affordable method for CD4+ T lymphocytes isolation and enumeration is needed. We describe a microfluidic affinity separation that isolates CD4+ T lymphocytes from blood samples. Processed blood samples were injected to a straight channel microfluidic device; specific monoclonal antibody and shear stress were used to capture CD4+ T lymphocytes on the affinity surface while elute background cells. The fluorescent conjugated antibodies were used to stain and visualize corresponding cells. Captures were observed using inverted epifluorescence microscope with appropriate filters. Images were analyzed using ImageJ software. After separation, CD4+ T lymphocytes were isolated with high purity and efficiency. This method is inexpensive, rapid, and effective to isolate and enumerate CD4+ T lymphocytes from blood samples. It enables downstream analysis of CD4+ T lymphocytes.

Keywords: Bioanalytical, Lab-on-a-Chip/Microfluidics, Separation Sciences

Application Code: Bioanalytical

Methodology Code: Microfluidics/Lab-on-a-Chip
Macroaffages are immune cells that are known to produce large amounts of nitric oxide (NO) when endotoxins are present. However, pro-inflammatory and anti-inflammatory phenotypes produce different amounts of NO and reactive nitrogen species (RNS). For instance, NO can react with superoxide to produce peroxynitrite, a highly reactive oxidant that has been linked to several neurodegenerative diseases. Previously, we reported a microchip electrophoresis with electrochemical detection method to detect NO in bulk cell lysates following stimulation with lipopolysaccharide. However, to study differences in RNS production due to cellular heterogeneity, a single cell analysis system is necessary. This work describes the progress in developing and optimizing a single cell analysis device to detect these species. First, macroaffages were loaded with 6-carboxyfluorescein diacetate (6-CFDA). Once inside the cell, the diacetate group is removed, forming 6-CF, which is a fluorescent and electrochemically active molecule that can be detected from the single cell lysate by both laser induced fluorescence and electrochemical detection. Then ascorbic acid was added to the media and absorbed by the cells. Ascorbic acid is oxidized at a low potential and can be selectively detected using electrochemical detection. Then 6-CF and ascorbate were detected in macroaffage cells using a combination of fluorescence and electrochemical detection to demonstrate the capability of the system. The ultimate goal is to use combined electrochemical and fluorescence detection to study the variations of RNS in macroaffage cells due to different cellular phenotypes.

NIH, NSF, and the Madison and Lila Self Graduate Fellowship are acknowledged for their support.

Keywords: Capillary Electrophoresis, Electrochemistry, Lab-on-a-Chip/Microfluidics, Separation Sciences
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Complete blood counts and chemistry panels are routine clinical diagnostic tests on whole blood where a variety of blood parameters indicative of a patient’s health status are measured. Three important examples include: hematocrit percentage, total protein, and albumin concentrations. The importance of each ranges from a sign of internal bleeding, a first indicator of multiple myeloma, and an overall nutritional marker, respectively. The ability to obtain these quantitative clinical measurements from a small aliquot of blood in a rapid and cost-effective manner is critical to point-of-care applications. With the continued advancement of microfluidic technology, microdevices have become attractive solutions for clinical diagnostic measurements. In the presented work, polyester transparencies are laser-printed and cut in order to fabricate 5-layer microfluidic discs that analyze hematocrit, total protein, and albumin measurements from a few drops of blood. These devices are simple, disposable, cost-effective, and made from easily accessible materials and fabricated with standard lab/office equipment. Utilizing centrifugal microfluidics, rotation-driven fluid flow is facilitated through the microchannel architecture, allowing for whole blood separation, plasma metering, and mixing. Both colorimetric and image analysis using a cell phone camera and custom application have led to calibration curves with excellent R2 values (0.999) and clinically relevant dynamic ranges. Preliminary studies have resulted in hematocrit values within 8% of clinically validated measurements, while total protein and albumin values are within 15%. Continued research is underway to increase the close correlation between the data obtained utilizing the presented devices and other conventional methods.

Keywords: Clinical Chemistry, Lab-on-a-Chip/Microfluidics, Method Development, Protein

Application Code: Bioanalytical

Methodology Code: Microfluidics/Lab-on-a-Chip
Circulating tumor cells (CTCs) expressing invasive phenotypes down-regulate epithelial antigens such as EpCAM. As such, populations of CTCs may have a continuum of phenotypes. We have used a CTCs selection strategy that employs a panel of antigens to improve the clinical sensitivity of the CTC assay. In addition to EpCAM, we used FAP antibody to target CTCs. Cells expressing FAP are found in >90% of human epithelial cancers. A dual selection process was used that employs two microfluidic chips for CTC\textsuperscript{FAP} and CTC\textsuperscript{EpCAM} arranged in a serial fashion so each sub-population can be individually analyzed. The CTCs selection chips were made from a thermoplastic via hot embossing and provide high CTCs recoveries and purities >85%. Using a detection threshold at the 99% confidence level, clinical sensitivities of 100% for metastatic ovarian, colorectal, prostate, pancreatic, and 80% for breast cancer were achieved. Molecular analysis of both sub-populations of CTCs was performed. Gene profiling showed that CTC\textsuperscript{EpCAM} from pancreatic cancers demonstrated overexpression of CD133, CD24 and CD44 mRNA, which are markers indicative of a cancer stem cell characteristic. CTC\textsuperscript{FAP} showed high expression of CD133, CD24 and CXCR4, but no CD44. KRAS mutational analysis using a PCR/LDR assay detected mutations in codons 12 in 80% of CTC\textsuperscript{FAP} and CTC\textsuperscript{EpCAM} samples isolated from pancreatic and colorectal cancer patients. In few samples, CTC\textsuperscript{EpCAM} tested negative for KRAS mutations, but CTC\textsuperscript{FAP} from the same patient showed mutated gene. This is important finding useful in decision making process on EGFR-targeting therapy in cancer patients.
Microscopy Techniques for Biomedical and Pharmaceutical Applications

Toxicity Evaluation of Doxorubicin Loaded Poly(butylcyanoacrylate) (PBCA) Nanoparticles Using In Vitro and In Vivo Models of the Blood Brain Barrier

The interaction between Polysorbate 80-coated Poly(butylcyanoacrylate) nanoparticles (PBCA NPs) loaded with doxorubicin and without doxorubicin with in vitro models of the blood brain barrier (BBB) was evaluated using Langmuir Blodgett monolayer techniques, atomic force microscopy and lactate dehydrogenase measures of cell membrane toxicity. Results indicate that PBCA NPs loaded with doxorubicin disrupt the composition of the liquid condensed (LC) and liquid expanded (LE) phases of the 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) lipid monolayer. The disruption is concentration dependent and increased for PBCA NPs with doxorubicin. Lactate dehydrogenase (LDH) assays using a primary astrocyte and brain capillary endothelial cell (BCEC) culture model of the BBB indicate that PBCA NPs containing doxorubicin exhibit higher levels of toxicity than those lacking doxorubicin. This approach provides valuable information to assess nanoparticle toxicity for drug delivery to the brain.

Keywords: Bioanalytical, Biotechnology, Lipids, Microscopy

Abstract Text

Abstract # 1810-1

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Date: Wednesday, March 11, 2015 - Morn
Time: 08:30 AM
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Understanding molecular transport processes in nanoconfined environments is important for a lot of applications including separation, sensors, heterogeneous catalysis, and drug delivery. It was recently found that the axial motion of individual biomolecules/nanoparticles inside nanopores can be significantly slower than in bulk solution, suggesting that either chromatographic adsorption was present and/or the viscosity inside the nanopores was unusually high. In this study, we measured the 3D trajectories of individual nanoparticles diffusing in cylindrical alumina nanopores using an astigmatism-based 3D single molecule/particle tracking method that was recently developed in our group. C18 solutions of a series of concentration and sucrose were used to modify surface properties and control viscosity, respectively. With the ability to track particle precisely within a range as large as 4 µm, we were able to observe different motions of nanoparticles for a relatively long period, including diffusion in single pore, multiple pores and across adjacent pores. We found that the trajectories, diffusion coefficients and mean-square displacement (MSD) plots varied with C18 percentage and viscosity of media, potentially providing a method to distinguish the effects of adsorption and viscosity. These observations provide new insights into conventional liquid chromatography as well as size exclusion chromatography and membrane separations. An upright Nikon Eclipse 80i microscope is used for single particle fluorescence imaging and a cylindrical lens is introduced to obtain axial information. All data is analyzed by NIH ImageJ and Matlab.

This work is supported by NCSU startup to G.W.

Keywords: Chromatography, Fluorescence, Membrane, Microscopy
Application Code: Pharmaceutical
Methodology Code: Microscopy
A beam-scanning microscope based on Lissajous trajectory imaging is described for achieving streaming 2D continuous ~1kHz imaging or 3D continuous video rate volume mapping. The microscope utilizes two fast-scan resonant mirrors to direct the optical beam on a circuitous trajectory through the field of view to generate a 2D image. By incorporating a resonant Z-scanning assembly, sample volume mapping can be achieved. The separation of the full Lissajous trajectory time-domain data into sub-trajectories (partial, undersampled trajectories) affords effective frame-rates much higher than the repeat time of the Lissajous trajectory. By using a model-based image reconstruction (MBIR) 3D in-painting algorithm to interpolate the missing data for the unsampled pixels, full images are recovered. The MBIR algorithm uses a maximum a posteriori estimation with a generalized Gaussian Markov random field prior model for image interpolation. Because images are acquired using photomultiplier tubes or photodiodes, parallelization for multi-channel imaging is straightforward.

Preliminary results (Figure 1) for 2D Lissajous trajectory imaging demonstrate experimental frame rates of 1.460 kHz for simultaneous acquisition of laser transmittance and polarization-dependent second harmonic generation (SHG). Multi-channel data acquisition cards allow for simultaneous acquisition from multiple detectors, for perfect image overlay. The high frame rates also successfully removed image blur due to sample motion. Preliminary results for 3D volume imaging show that, in combination with the MBIR in-painting algorithm, this instrument has the ability to generate video rate images with the potential to access 6 total dimensions of space, time, and polarization for SHG, spectral TPEF, and laser transmittance data.

Funding acknowledged from NIH, the Henry B. Hass Fellowship, and the Showalter Foundation.
Microscopy Techniques for Biomedical and Pharmaceutical Applications

Label-Free Measurement of Cell-Gold Cleft Gap Distance Using Surface Plasmon Microscopy

Biointerface has been attracting considerable attention in variety of areas including medical or bioanalytical applications. In extracellular recording, cell-electrode interfaces are important to have tight contact of cells on the electrodes since it strongly influences the signal intensity, and thus a tool for its investigation is highly required.

Here, we introduce new surface plasmon microscopy (SPM) allowing label-free and real-time investigation of a biointerface, especially measurement of cell-electrode cleft gap distance, which was difficult with conventional fluorescence or electron microscopy. The microscopy exploits surface plasmon polaritons, electromagnetic waves propagating along a metal-dielectric interface, and combines two different modes; one is for real-time large area observation of the cell-gold interface (lens-imaging-type, LISPM); the other is for in situ measurement of the gap distance with high spatial resolution up to the diffraction limit (scanning localized, SLSPM).

Human embryonic kidney (HEK) 293 cells cultured on poly-L-lysine coated gold substrates for 3-6 days in vitro were observed with the SPM. LISPM qualitatively visualized the cell adhesion on the surface (Fig. 1a) and SLSPM quantitatively determined average distance of 49.6±15.9 nm (Fig. 1b), which is in good agreement with the previously measured value (37 nm) with transmission electron microscopy (TEM) (G. Wrobel, et al., J. R. Soc. Interface, 5 (2008) 213.). In conclusions, the developed SPM could be a powerful technique to pave the way for further understanding and optimizing the interface.

Acknowledgements:
K. Toma acknowledges the support provided by the Al-exander von Humboldt Foundation.

Keywords: Biological Samples, Biomedical, Microscopy, Nanotechnology
Application Code: Biomedical
Methodology Code: Microscopy
Bioanalytical methodology has seen major advancements since the introduction of microfluidics. However, rapid and selective protein quantitation in continuous flow systems with a simple optical readout remains an unmet challenge. For example, to study hormone secretion from endocrine cells, microfluidic devices with on-chip, low dead-volume valves are particularly well suited. Although microfluidic systems with on-chip valves are ideal for secretion sampling from small numbers of cells, the development of compatible, simple-readout assays has lagged behind device development. Here, we have combined a customized nanoliter rotary mixer with our novel proximity immunoassays, which permit selective, direct-readout fluorescence quantitation of proteins in picoliter-to-nanoliter volumes. The device contains low dead-volume, push-up membrane valves for precise sample and proximity probe metering (9 nL each), peristaltic pumping, and rotary mixing. This integrated, sample-to-readout system allows continuous sampling, mixing times as short as 2.2 s, then detection of only 180 attomoles of thrombin and we expect similar results for the insulin.

**Abstract Text**

**Keywords:** Bioanalytical, Microscopy, Protein, Quantitative

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Microscopy Techniques for Biomedical and Pharmaceutical Applications

Analysis of Pharmaceutical Crystallization by Video Rate Polarization-Modulated Second Harmonic Generation Ellipsometric Microscopy

The crystalline form of pharmaceutical materials can profoundly impact the bioavailability and materials processing characteristics. Consequently, screening for new crystal forms has become a critical step preceding formulations design. Standard methods based on X-ray diffraction become challenging as the throughput increases and the volume of crystalline material per trial decreases. Polarization-dependent second harmonic generation (SHG) microscopy is suggested as a high-speed complement to current methods for initial assessments of crystal form. As a first step toward this application-space, instrumentation for rapid polarization modulation SHG microscopy is demonstrated, capable of completing full polarization acquisition at each pixel in a 315×315 image with up to video-rate frame rates. Alternative established methods of polarization modulation are based largely on mechanical rotation of polarization optics. These methods are relatively slow, introducing 1/f noise as well as artifacts from sample movement, bleaching and thermal effects, and are incompatible with high-throughput assays. In the present work, polarization modulation was performed by cycling through a series of 10 unique input polarizations at 8 MHz using an electro-optic modulator. Rapid polarization modulation was combined with fast beam scanning and synchronous digitization to enable nonlinear optical stokes ellipsometric (NOSE) microscopy at video rate. Each video rate frame provided a set of 10 polarization dependent images per detector, which were converted using linear fitting to a set of 5 parameters that are directly related to the Jones tensor of the sample and provide information on crystal form and orientation. Preliminary measurements designed to assess the advantages and limitations of NOSE microscopy for high-throughput detection of new crystal forms suggest promise for this approach.

Funding was provided from the NIH Grant Numbers aR01GM-103401 and R01GM-106484 from the NIGMS.

Abstract Text

Materials Characterization, Microscopy, Pharmaceutical, Ultra Fast Spectroscopy

Pharmaceutical

Microscopy
Microscopy Techniques for Biomedical and Pharmaceutical Applications

Particle ID Robots - Design and Application of Image Directed Raman + LIB Spectroscopy

The first Raman LIBS (Laser Induced Breakdown Spectroscopy) combination system characterizes micro particles, and has many industrial and medical applications.

Firstly, the system determines particulate morphology and size. Particles within a size range of 2µm to several millimeters can be analyzed easily. After this step, the system locates an exact point on each particle where the micro Raman and/or LIBS will be applied to reveal its composition.

Raman spectra exhibits features specific to molecular structure, giving a 'fingerprint' for comparing and differentiating materials.

The LIBS technique utilizes a high energy pulse laser to ablate a microscopic amount of material and raise its temperature by tens of thousands of degrees. As the various elements return to their ground states, excited matter emits characteristic emission spectra that can be measured. The specialty of the instrument is its ablation area of 20µm in diameter, and its high quality spectroscopic system. This technique enables the system to reveal the chemical elements of a particle larger >20µm with a single shot.

These laser systems, coupled with advanced image analysis techniques provide maximal information about the micro-particle. Chemometric algorithms subsequently match the spectroscopic fingerprints of micro-particles with a spectra library.

Benefits:

- easy, fast and self-contained □ high throughput particle ID of unknown or contamination materials
- parameters are software controlled in receipts □ high repeatability and reproduce-ability
- identification with real samples □ immediate ID of materials from customized libraries

Application of this new technology

We explain the instruments application in the pharmaceutical and automotive industries. See how any user can match the chemical fingerprints of contaminants, allowing their identification. Useful decisions regarding the damaging potential of the particles or their general avoidance can be made immediately.

Keywords: FTIR, Pharmaceutical, Quality Control, Raman
Application Code: Quality/QA/QC
Methodology Code: Microscopy
Dynamic Imaging Particle Analysis (DIPA) is increasingly being used as a method of characterizing different particulate types in heterogeneous samples. DIPA can measure many different particle properties simultaneously for each particle, which can be later used to construct digital “filters” to separate out each different particle type. Many different filtering methods are available, the simplest and most common being value filtering, like one would do in a spreadsheet. In value filtering, a range of a single measurement, or of multiple measurements, is defined and the particles that fit those criteria are counted. There are also statistical filters, which use particle statistics to search for particles which have similar statistics to a set of user-designated particles. This is more generally known as pattern recognition. Even within statistical filtering, there are many different algorithms available.

All of these types of digital filters produce results which may contain false positives and false negatives. The only way of measuring how well a filter performs is to actually compare the results to a “hand classified” result. By hand classified, we mean that each particle has been looked at and classified by a human being, as they would in manual microscopy. Obviously, this can be a very tedious process. A method has been developed to more rapidly put together such a comparison in order to quantify the effectiveness of different filters attempting to isolate the same type of particle. This presentation will outline both the method, and results of using the method on a specific sample of data. From this analysis, some general conclusions can be drawn which will be useful in narrowing down the most efficient methods for particle identification.
For flour supplied to the baking industry, various properties are of particular concern including but not limited to endosperm purity, protein content, and particle size. Of these properties, flour purity in terms of endosperm content is of higher interest; however, traditional methods have been limited to measurement of the impurities by ignition of the organic material and weighing the mineral residue. Factors such as endosperm purity, protein content, and particle size need to be monitored closely by millers in order to meet specifications of the baking industry because they have a significant impact on the value of the final product for end use. Near infrared chemical imaging enables the identification of flour composition on a pixel-by-pixel basis and subsequent pixel counting yields a quantitative result. From select spectral characterizations, different factors such as protein content and endosperm purity can be measured directly. Furthermore, image analysis from the same experiment can be used to determine particle sizes within the heterogeneous mixture. Imaging data are presented to demonstrate the utility of quantitative chemical imaging in the milling field.

Keywords: Imaging, Near Infrared, Process Analytical Chemistry, Quantitative
Application Code: Quality/QA/QC
Methodology Code: Near Infrared
Lebel and coworkers have recently shown that triazine derivatives are a class of small organic molecules capable of forming an amorphous phase without requiring a deep quenching from the melt or an ultrafast solvent evaporation. The outstanding glass-forming ability (GFA) of these molecular glasses is attributed to the poor packing efficiency of their self-assembled aggregates that are held by multiple hydrogen bonds. To deepen our understanding of the relationship between the H-bonds and the observed GFA, we aim to quantitatively monitor hydrogen bonding while these compounds undergo their glass transition (Tg), from their viscous state to their vitreous one.

In this presentation, we will show that variable temperature infrared spectroscopy is a tool of choice to probe the vitrification process of these glass-formers. Indeed, we succeed to correlate their molecular features to their bulk thermal properties by pairing the selectivity of this vibrational technique with chemometrics analysis, leading to the quantitative monitoring of hydrogen bonding during the glass transition. For instance, this approach reveals that, on average, around 60% of the molecules are still H-bonded even at 50 °C above the Tg of the molecular glasses, preventing their reorganization into an organized lattice. These results underline the significant role of hydrogen bonding in frustrating the crystallization upon cooling, providing new insights to further our knowledge of glass engineering.

**Keywords:** Characterization, Chemometrics, FTIR, Vibrational Spectroscopy

**Application Code:** Materials Science

**Methodology Code:** Vibrational Spectroscopy
When choosing a near-infrared spectrometer (NIR) for industrial applications, the question of which technology is best should be considered. The choice often times comes down to dispersive scanning grating spectrometers (SGS) versus Fourier transform near-infrared spectrometers (FT-NIR). This discussion will review a comparison of the two techniques as they apply to the analysis of clear liquid samples, such as the hydrocarbon liquids found in petroleum and chemical industry applications. In addition, the comparison will be narrowed to include instruments that are coupled to the sample probe/cell through fused silica fiber optic cables. This will explain the basic differences between the various instrument types and how this relates to performance for an on-line analyzer system. A discussion of the true advantages that each technology holds will be provided.
The safety of our food supply depends heavily on the quality and purity of ingredients used to produce food products. Adulteration of food and feed ingredients, whether economically motivated, contamination with other substances, or simply off-spec products impact product safety, quality and consumer perceptions of the product. Many companies invest considerable resources in high-end analytical instruments and persons to operate them in an effort to detect individual contaminants and adulterants. Smaller companies often do not have those resources available in-house due to the cost of instruments and the personnel to operate them.

FT-NIR has been employed by the pharmaceutical industry for raw material identification for many years. The same information can be easily extended to use FT-NIR as a non-targeted screening method for food and feed ingredients. Non-targeted screening is intended to identify ingredient shipments whose NIR spectra differ from what is normally received. When an ingredient batch has been determined to differ from previous shipments that have met established quality and safety criteria it can be excluded from the production queue. The suspect ingredient batch can then be examined using more targeted analytical methods. Statistical evaluation of NIR spectra can be used to determine variation in ingredients between individual lots of materials. These statistical methods can be used to determine if the NIR spectrum for a food ingredient is within the normal variance seen for that particular ingredient. In this paper the use of FT-NIR as a non-targeted screening method for food and feed ingredients will be presented. Two different software methods for determination of variation between individual lots of ingredients will be described including method development and validation considerations. Applications that can be successfully addressed will be discussed along with limitations of the technique.

Keywords: Food Contaminants, Food Identification, Food Safety, Near Infrared
Application Code: Food Safety
Methodology Code: Near Infrared
Wavelength standards for high resolution near infrared (NIR) spectroscopy are excellent. The same cannot be said for low to moderate resolution instruments. The development of NIST's SRM2035 and other rare earth standards have been a significant improvement in the last decade. However, problems and inconsistencies still exist in these standards. We will review the state-of-the-art and present data on the temperature dependence of the SRM2035 peaks. In addition we will investigate the utility of using atomic emission lines, specific standard laboratory solvents, and other rare earth glass materials for wavelength calibration and calibration transfers. A technique for using spectroscopy to measure the temperature of a rare earth filter and then correct the peak wavelengths in real time will be presented.

Keywords: Calibration, Near Infrared, Spectroscopy, Standards
Application Code: Other
Methodology Code: Near Infrared
In durum production, the objective is to produce a semolina product that is uniform in color and particle size and free from non endosperm material. The presence of the latter item is revealed via visual speck counting under a low power microscope. The recently developed quantitative near infrared imaging based on chemical distinction provides the approach to objectively rank the purity of various durum milling fractions. The objective is to enable operating millers to optimize their processing parameters at key unit process steps based on chemically determined objective numerical data.

Keywords: Imaging, Near Infrared, Quality, Quantitative
Application Code: Agriculture
Methodology Code: Near Infrared
## Head Space Raman Spectroscopy

Head space Raman spectroscopy is a method well suited to the study of compounds in the vapor phase. We have used it to study the volatility of solvents, the release of gas dissolved in a liquid solvent, and the difference in molecular interactions in the vapor phase vs. the condensed phase (liquid or solid). An advantage of head space Raman measurements is that spectra of the condensed and vapor phases can be obtained from the same sample. The differences between the Raman spectra of the liquid and vapor phases can be quite striking; e.g., the effect of hydrogen bonding on the OH stretch of methanol vanishes in the vapor phase. We will present the results of our studies of phase dependent molecular interactions, gas release from solution, solvent volatility, and partitioning of mixed solvents.

**Keywords:** Materials Characterization, Raman, Separation Sciences, Spectroscopy  
**Application Code:** Materials Science  
**Methodology Code:** Near Infrared
In order to produce consistent high quality plastic bottles it is essential to verify the crystallinity of blown bottle walls in order to ensure product consistency, strength and overall quality. Current methods such as X-ray diffraction, density measurement or calorimetry are often inaccurate, slow and require chemical consumables.

In this paper we present an alternative method, using FT-NIR, for the performance of rapid determination of bottle crystallinity that overcomes these limitations.

**Keywords:** Beverage, FTIR, Molecular Spectroscopy, Polymers & Plastics

**Application Code:** Polymers and Plastics

**Methodology Code:** Near Infrared
Synthetic polymers manufactured by a condensation process such as polycarbonates may have a significant fraction of low molecular weight oligomers. This oligomer fraction can have an effect on the overall material properties such as melt flow rate, impact resistance and tensile strength. Short oligomers may also cause aesthetic issues during the injection molding process by creating deposits on the mold surface. Accurate characterization techniques are required to determine the types and amounts of oligomers generated during polymerization to aid in the development of appropriate process controls.

For condensation polymers, the oligomer fraction is typically measured using chromatographic techniques such as size exclusion chromatography and high-performance liquid chromatography (HPLC). Both of these techniques can provide for reproducible measurement of the total amount of oligomers present however identification of individual oligomers can be problematic. Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry is now an established technique for identifying oligomers in many types of synthetic polymers. In this work, we investigate the correlation between the relative signal areas from both HPLC and MALDI analyses of oligomer fractions for a variety of polycarbonate and polyetherimide resins. The techniques are found to be quite complementary and provide for a more complete characterization than can be achieved using a single technique.
Mammalian olfaction possesses a high capacity for the discrimination and quantification of gas phase compounds as well as the ability to identify new compounds and mixtures. While there have been significant advances in machine vision through the invention of the coupled charge device (CCD) chip, its equivalent for sensing the chemical environment has yet to be achieved. One of the biggest challenges has been the integration of chemically diverse sensing materials (the equivalent of olfactory receptors) onto integrated circuits. Current approaches for generating chemically diverse sensors include conducting composites containing different polymers that function through variations in analyte partitioning and corresponding changes in resistance. While these systems have excellent performance, fabrication methods are not compatible with lithographic processing of integrated circuits and spatial deposition control during post chip fabrication is very limited.

The electrodeposition of conducting polymers has the potential of overcoming spatial limitations, however the number of polymers available are few resulting in limited chemical diversity. In this presentation an approach using co-electrodeposition of conducting polymers and chemically diverse co-monomers is explored as a means for enhancing diversity in conducting polymer composites that will be compatible with integrated circuits with sensing elements on the micron and sub-micron scale. In particular results from the electrochemical copolymerization of pyrrole and styrene derivatives (styrene, 4-m-styrene, 4-chloromethylstyrene and 4-t-butoxystyrene) will be described. The ability to tune the relative amounts of the co-monomers in the resulting polymer as a function of polymerization potential and its impact on sensing will be covered.

Keywords: Array Detectors, Data Analysis, Electrochemistry, Polymers & Plastics
Application Code: Polymers and Plastics
Methodology Code: Electrochemistry
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Abstract Text

There is a growing interest for two-dimensional chromatography (2D) including its application to polymers. This is motivated by the need to characterize more complex materials such as multicomponent blends and copolymers with tailored microstructure. This is especially important in order to establish the relationship between structure and property, to gain a better understanding of the reaction pathways, and also for protecting intellectual property. 2D consists of coupling a first dimension where separation is carried out based on one material property (e.g. composition) with a second dimension where separation is based on a second material property (e.g. molecular weight). In this presentation, recent advances in 2D chromatography will be reported such as the use of UHP-SEC in the second dimension and coupling with infrared spectroscopy detection. Several examples of application of two-dimensional chromatography will be reviewed that will illustrate the benefits of using this technique in an industrial R&D environment.

Keywords:  
HPLC, Liquid Chromatography, Materials Characterization, Vibrational Spectroscopy

Application Code:  
Polymers and Plastics

Methodology Code:  
Separation Sciences
The volume phase transitions (VPT) of hydrogels are often utilized for chemical sensing applications. Our group has developed two dimensional photonic crystal (2D-PC) hydrogels to monitor VPT in response to stimuli by attaching a 2D photonic crystal array of ~500 nm polystyrene particles to the hydrogel surface. The particle spacing of the attached 2D photonic crystal array varies with the hydrogel volume. This volume can be measured by using a laser pointer to excite the Debye ring diffraction from the attached 2D array.

Decreasing the hydrogel thickness and/or the crosslink density increases the sensitivity of the sensor, but mechanically weakens it making it difficult to study. We developed a sacrificial polyvinyl alcohol (PVA) substrate to transiently ruggedize the 2D-PC sensors. To demonstrate the utility of the sacrificial substrate, we fabricated pH sensors that contain acrylic acid, acrylamide, and low concentrations of bisacrylamide crosslinker. These sensing gels were then attached to a PVA hydrogel substrate formed through a freeze-thaw process. The resulting bi-layer system is easily handled and shows long term stability. The PVA substrate was dissolved by transient heating to 70°C. The released pH sensing gels show dramatic swelling as the pH increases. Sacrificial substrates will enable the use of super-responsive 2D-PC sensors.

This work was funded by the Defense Threat Reduction Agency (DTRA) grant no. 1-10-1-0044

Keywords: Polymers & Plastics, Sensors, Spectroscopy
Application Code: Polymers and Plastics
Methodology Code: Sensors
Various novel colored poly(urea-urethanes) have been prepared by using a variety of benzothiazole derivatives, 3-amino phenol and hexamethylene diisocyanate. The resultant colored poly(urea-urethanes) were characterized by elemental analysis, IR, thermogravimetry. All the poly(urea-urethanes) were applied on polyester fabrics. These dyes were found to give a wide range of colored shades with very good depth and levelness on polyester fabric. The dyed fabric showed good fastness to light and very good to excellent fastness to washing.
Tetracyclines are commonly used antimicrobial agents for agricultural livestock. Increased use of antibiotics in animal feed to enhance the growth of livestock has historically shown negative impacts on human health. Issues related to antibiotic resistance following consumption of contaminated animal muscle and milk have been fully documented within global populations. Regulating the agricultural use of tetracyclines is quickly becoming a global concern heading for more strict regulation and frequent testing. It is important to guarantee that beef, chicken, and milk are safe to eat within the Maximum Residue Limits (MRL) allowable by the Codex Alimentarius Commission, the responsible body for establishing international food standards for public safety and fair food trade.

With regulation changes, many laboratories will see increases in the number of samples and methods to test. Many food safety methods used for testing of agricultural contaminants in foods do not utilize current automation technology or improved chemistries to streamline the typical laboratory workflow that would greatly reduce the impact of increased testing and remove potential bottlenecks. Improving established methods to reduce the risk of backlog and human error is essential for timely reporting of results with confidence in the data. To help address the current global focus on the extensive and increasing agricultural use of tetracyclines, new methods using simple automation and new chemistries and technology will be utilized to accurately identify and consistently detect tetracyclines at the MRL of 100 µg/l in cattle milk, and 200 µg/kg in both cattle muscle and chicken muscle.
Solid phase microextraction (SPME) is a solventless, fast, easy and relatively inexpensive sample preparation technique that integrates sampling, extraction and preconcentration into one step. It has widespread applicability in various fields that include environmental, food, drugs, in-vivo analyses etc. Commercial coatings may be expensive, show relatively short lives, extract limited numbers of compounds, and have relatively low thermal and mechanical stability, and/or solvent incompatibility. The aim of this study is to develop an SPME device that is thinner, robust, long lasting, has higher mechanical strength, greater thermal and solvent stability, and is devoid of the other drawbacks of commercial coatings. Silica-based nanoporous coatings were prepared via sputtering of high purity silicon onto a fiber substrate, which resulted in porous structures. The thicknesses of these fiber coatings were around 1 [micro]m. To increase the density of –OH groups on the surfaces, the coatings were treated in piranha solution and then reacted with n-octadecyldimethylmonomethoxysilane to render the surfaces hydrophobic. Using silanol/silane chemistry, we can introduce other functional groups for desired selectivity. Sputtering provides good control over coating thickness and high reproducibility. We compare our fibers against commercial PDMS 7 [micro]m fiber for the extraction of saturated alkanes, primary alcohols, and various real life samples. Preliminary results show that despite having a thinner coating, our fibers have comparable performance, and in some cases, especially for higher molecular weight compounds, show better results than the commercial fiber.
Following the procedure presented by Grimm et al. (2000), catfish was heated in a modified microwave oven while being purged with N\textsubscript{2} flow. Water and flavor chemicals condensed in a cold trap and were analyzed by Solid Phase Microextraction/Gas Chromatography/Mass Spectrometry. Figure 1 shows the oven and customized glassware (Widget Scientific, Inc., Baton Rouge, LA, USA). A standard kitchen-type 1000 watt microwave oven was modified by drilling 2 holes and attaching lengths of conductive pipe to act as chokes. Chokes prevent microwave leakage, which can be verified by a microwave detector.

Three parameters influence the transfer efficiency of volatile flavor chemicals to the condensate: N\textsubscript{2} flow, microwave power and heating time. These parameters are interdependent. Response Surface Methodology (RSM) based on a central composite design (CCD) is a statistical technique which efficiently determines the optimum combination of these interdependent parameters. Preliminary experiments were performed to determine reasonable ranges and 5 levels of each parameter were analyzed in 15 combinations. Each combination of parameters was applied to a 20 g portion of catfish which had been spiked with 5 ppb of 3 chemicals: Methylisoborneol (MIB), Geosmin (GSM) and Decahyndronaphthol (DHN). MIB and GSM are the most common off-flavors occurring in catfish. DHN is used as an internal standard. After analysis by GC/MS, peak areas were totaled. The CCD was analyzed by RSM. When the analysis of variance for the model is significant and the lack of fit was not significant, the model is valid.

Commercially available microwave ovens come in a range of power levels. Different glassware configurations can be used and many different food matrices can be analyzed. For each combination of variables, RSM must be repeated in order to determine the optimum combination of parameters.

We repeated this procedure using a condenser between the purge vessel and receiving cylinder and using vegetable oil spiked with the same analytes. The prediction profiler was used to determine each parameter value yielding the highest recovery. RSM is a powerful and efficient method for determining the optimum levels of interdependent parameters.

Mycotoxin testing of raw agricultural products and food stuffs is an essential part of most country’s food safety programs, ensuring the levels present are within the permissible limits per country or by the Commission Regulation (EC) No 1881/2006. Aflatoxins are the most widely tested mycotoxins, presenting challenges to identify this mycotoxin quickly and accurately as global food trade continues to rise.

AOAC methods for aflatoxin B1, B2, G1, G2 testing utilize post-column derivatization that requires iodine and a reaction coil. Researchers have shown post-column derivatization is successful using a post-column photochemical reactor enhancement detection system (PHRED). In both situations, specialty equipment is required. In addition, the fluorescence wavelength chosen for analysis varies among published research on aflatoxins HPLC analysis without discussion on sensitivity, linearity, repeatability of standards, as well as recovery and reproducibility of a variety of problematic sample matrices across a range of fluorescence wavelengths. A unique and innovative methodology has proven that pre-column derivatization is an effective means for generating acceptable linearity of aflatoxin B1, B2, G1, G2. Optimizing thermal conditions, in combination with the most sensitive fluorescence emission wavelength, generates reproducible and linear results over the course of several days of testing. The ability to generate accurate data with standards and problematic sample matrices is obtainable using commonly available laboratory equipment to allow for simplified but reliable routine testing of aflatoxin B1, B2, G1, G2 that conforms to the EU performance criteria with the highest sensitivity and without specialized hardware or procedures.

**Keywords:** Agricultural, Food Contaminants, HPLC Detection, Sample Preparation

**Application Code:** Food Contaminants

**Methodology Code:** Sampling and Sample Preparation
Quantitative identification of engineered nanomaterials (ENMs) is complex, as these materials often exist at low particle number, very low mass concentration and are highly dynamic. ENMs may be found in environmental, biological, or engineered solutions as dissolved ions. In the simplest analytical cases (e.g. filtration then total metal analysis by ICP-MS), only total element concentrations are measured, usually at higher than environmentally relevant concentrations, and thus no “nano-specific” qualities are determined. In other cases, particle measurements are obscured by dilute particle number in complex matrices (e.g. difficult to “find” by TEM or Nano Tracking Analysis) or only broad particle distributions are possible (e.g. when measured by DLS, UV/VIS, etc.). Ideally, all comprehensive ENM studies should implement multiple characterization techniques, but often only a fragment of data is obtained because of these analytical difficulties and using multiple techniques can be time consuming, require much expertise, and expensive to obtain instrumentation. However, single particle (SP)-ICP-MS may replace many standard analytical analyses; either as a fast screening technique to determine the presence, size and concentration of nanomaterials in varied solutions and/or be implemented to collect precise data to monitor changes of ENMs and associated dissolved metal in time resolved environmental studies, all at dilute (ng/L) concentrations. A few varied studies are highlighted here, which use SP-ICP-MS to monitor dissolution of Ag ENM in environmental samples and release of NMs from textiles after washing. Increased development in terms of both data processing software and instrumental hardware have come about in recent years, which will facilitate SP-ICP-MS being implemented more easily in many laboratories. Discussed in this context, SP-ICP-MS can provide as much or more information in a faster and easier than several current analytical techniques combined.

Keywords: Environmental Analysis, Environmental/Biological Samples, ICP, Mass Spectrometry
Application Code: Nanotechnology
Methodology Code: Mass Spectrometry
Spectroscopic Characterization of Nanomaterials

Thermal Analysis Couples with Spectroscopic Techniques for Studying Aging of Coated Nanoparticles and Characterizing Interaction of Nanoparticles with Organic Pollutants

Evaluating the environmental and human health implications of nanomaterials requires an understanding of their fate, transport and transformation during their lifecycle, including their interaction with biological and other organic pollutants. Thermogravimetric analysis (TGA) and TGA coupled with infrared spectroscopy-GC-MS are used to investigate nanoparticle interaction with biomaterials and their environmental transformations coated nanomaterials. The study also included investigating how hazardous organic compounds adsorbed to engineered nanoparticles. The techniques have shown to be promising in studying the partitioning of hydrophobic contaminants between nano-particles and water which is an important parameter for modeling the fate and transport of these contaminants in aquatic environment. The octanol-water partition coefficient is the ratio of the concentration of a chemical in octanol and in water at equilibrium. Octanol is an organic solvent that is used as a surrogate for natural organic matter, and partitioning of contaminants between the two phases helps to determine the fate of contaminants in the environment, such as to predict the extent of bioaccumulation of contaminants aquatic biomes animals. This study focuses on the aging of coated nanomaterials and the influence of nano-particles on the partitioning pollutants between water and organic phases. Sorption of hydrophobic organic pollutants to nano-particles increased the concentration of contaminants in the aqueous phase as compared to the “true” partitioning due to the octanol-water partitioning. The effects of nano-materials on the mobility of pollutants that are commonly found at many sites has been studied. These compounds including 2 and 3-ring PAHs: naphthalene, anthracene, and phenanthrene have relatively low water solubility and differing mobility in the soil.

Keywords: FTIR, GC-MS, Nanotechnology, Thermal Analysis
Application Code: Nanotechnology
Methodology Code: Other
The presence of volatile organic compounds in carbon nanomaterial products such as single wall carbon nanotubes, multi wall carbon nanotubes, graphene, and modified single wall carbon nanotubes can affect its performance. Proper sampling and characterization/quantitation of volatile components in highly adsorbent solids requires multiple analytical techniques. Volatile organic compounds are usually identified by measuring the mass spectra of the gaseous component evolved from the heated solid samples. This presentation illustrates the use of an integrated Thermogravimetric Analysis (TGA) coupled with Infra-Red (IR) coupled with Gas Chromatography Mass Spectrometry (GC-MS) using PerkinElmer TL-9000 transfer line for the complete and comprehensive characterization of residual organic compounds in carbon nanomaterials. TGA-IR is used to determine the temperature range over which the release of volatile components occurs. TGA-GC-MS analysis is used to identify each component separately by the use of a capillary column. Quantitative information can additionally be obtained by the sample mass loss in thermogravimetric analysis (TGA) and the peak area in the GC-MS.

Combining these different techniques into an integrated easy to use system provide users with comprehensive characterization of organic based nanomaterial.

Keywords: GC-MS, Infrared and Raman, Nanotechnology, Thermal Analysis
Application Code: Nanotechnology
Methodology Code: Other
The interactions of nanoparticles (NPs) with the cells have gained increasing attention since NPs have been widely applied to biomedical fields including imaging, diagnostics, cancer therapy, targeted drug and gene-delivery. It was reported that NPs can change the fluidity of cell membrane. However, to our best knowledge, there has been no direct evidence provided regarding to whether and how flip-flop is involved in NP-cell membrane interaction which may due to the lack of surface/interface-sensitive techniques to study the interfaces between NPs and cell membrane. In our group, sum frequency generation (SFG) vibrational spectroscopy with an intrinsic surface/interface sensitivity was employed to study the interaction of model mammalian cell membrane (dDSPC/DSPC lipid bilayer) with Au NPs of four different sizes using three different dose metrics based on mass, NP number and surface area. The results show that the flip-flop rates trend to increase with the increasing size of Au NPs based on same particle number and surface area while they are independent of the size of Au NPs with the same mass. We rationalized the experimental results with a concept of “effective surface area” that is the real contact surface area between Au NPs and model cell membrane and can be estimated based on proposed mathematical model. This study is considered as the very first but crucial step toward the full understanding of the mechanism how NPs interact with model cell membrane.

Keywords: Laser, Membrane
Application Code: Nanotechnology
Methodology Code: Other
Biodegradable and biocompatible poly[(R)-3-hydroxybutyrate-co-(R)-3-hydroxyhexanoate] (PHBHx) electrospun nanofibers were fabricated by collecting the fibers on the sharp edge of a rotary disk at an angular speed of 3500rpm. Traditional methods, including wide angle X-ray diffraction (WAXD) and transmission Fourier transform infrared spectroscopy (FTIR), were used to examine the crystal structure and chain conformation of a bundle of fibers. The results revealed the existence of a new strain-induced metastable \(-\)crystalline form in the macroscopically aligned PHBHx nanofibers, with the chains extended and adopting a planar zig-zag conformation.

In this study, we utilized AFM-IR, a new technique combining Atomic Force Microscopy (AFM) and Infrared Spectroscopy (IR), to look at both the structural and morphological details of a single electrospun nanofiber with a diameter of no less than 200nm. The results indicate that the structure of individual fibers is largely dependent on their sizes, and thinner fibers tend to contain more \(-\)crystalline form. Comparing the AFM-IR spectra with the spectrum obtained from conventional FTIR, one can speculate that the FTIR spectrum is the average of the spectra of a bundle of rotary disk aligned fibers with different diameters. In addition, the mapping of individual nanofibers with 50nm spatial resolution revealed an interesting “core-shell” structure of the fibers where for each fiber, the peripheral area contains much more \(-\)structure. Hence, AFM-IR is a powerful tool to investigate the chemical and crystalline structure of a single electrospun nanofiber.

The authors acknowledge NSF Delaware EPSCoR for the support on this project.
Self-assembly of anisotropic gold nanorods (GNRs) is attractive since it provides an additional way to tailor the optical properties of localized surface plasmon resonance (LSPR). To achieve an ordered GNR array with highly uniform and precise confinement, a template-guided convective assembly on predefined areas has been developed. However, a systemic study on the critical factors such as nanorod concentration, aspect ratio, surface charge and surface property of substrate is needed to fully control the quality of the assembly. Furthermore, tuning the interparticle distance, the number of assembled layers and assembly areas are critical to optimize the collective LSPR property in pursuit of maximal optical property in biomedical application. Here, we designed a hydrophilic-hydrophobic surface treatment of template substrate by depositing a thin layer of perfluorodecyltrichlorosilane (FDTS) on lithographically patterned glass. The wetability contrast of surfaces serves as a virtual barrier wall to confine the nanorod assembly in designated pattern. It was found that lower surface charge and higher dimensional identity render the vertical gold nanorod assembly more organized and uniformed. Nanorod concentration was critical for a full coverage of a given area in a monolayer fashion. As the size of the hydrophilic areas increased, a multiple layer fashion was preferred. In addition, the interparticle distance was precisely tuned by varying the ionic strength of solution, resulting in various local electric field enhancements by plasmonic coupling, which was validated by COMSOL simulation results. The orderly GNR array provides a new insight into the enhanced optical transduction of surface plasmon in a chip format.

Keywords: Lab-on-a-Chip/Microfluidics, Nanotechnology, UV-VIS Absorbance/Luminescence
Application Code: Nanotechnology
Methodology Code: Microfluidics/Lab-on-a-Chip
A Group of uniform materials based on organic salts (GUMBOS) include organic salts which exhibit the unique and tunable properties of ionic liquids in the solid phase. Nanoparticles derived from these organic salts, i.e., nanoGUMBOS, have been successfully used in a variety of applications including biological and analytical applications. Size-dependent optical properties of nanoGUMBOS is the key to widening these applications in optoelectronic devices. In this work, novel strategies for size-control of nanoGUMBOS are investigated and size effects on optical properties are assessed. This research focuses on two main aspects: the first is optimization of the synthetic procedures and the second is the measurement of nanoGUMBOS optical properties. Herein, nanoGUMBOS were derived from organic semiconductors and syntheses of these compounds were assisted using ultrasound in the presence or absence of soft templates. Characterization techniques such as transmission electron microscopy, UV-Vis and fluorescence spectroscopy were used to analyze the efficiency of the investigated strategies. Absolute quantum yields were also measured for these nanomaterials using an integrating sphere. Our results show that the size and aggregation level of nanoGUMBOS, and ultimately the optical properties can be tuned using different synthesis procedures. Our data implies also that the type of anion in the GUMBOS structure has a major effect on quantum yield values. As a result of this investigation, the efficient use of these nanomaterials in organic light emitting devices is examined in this context.

Keywords: Microscopy, Nanotechnology, Semiconductor, Spectroscopy
Application Code: Nanotechnology
Methodology Code: Molecular Spectroscopy
Electron exchanges (ETs) between small (< 2 nm), highly monodisperse, mixed-valent Au monolayer-protected clusters (MPCs) containing one or more ferrocene (Fc\textsuperscript{+1/0}) redox species in dry, solid-state films will be discussed. We previously observed that electron exchanges in films of highly monodisperse Au\textsubscript{144} and Au\textsubscript{25} MPCs is charge-state and core-size dependent at both high and low temperatures. At reduced temperatures (down to 77 K), the thermally activated ET process dissipates, revealing non-Arrhenius behavior, as ET rates become independent of temperature. The present experiments show that the addition of mixed-valent ferrocenated thiols in the organothiolate ligand shells of Au\textsubscript{225}, Au\textsubscript{144}, and Au\textsubscript{25} impedes electron exchanges between MPCs at and below room temperature. As with the non-ferrocenated Au MPCs, the thermally-activated ET process is diminished at lower temperatures, and the ET rates become temperature-independent. Obliging ET to occur through the Fc\textsuperscript{+1/0} redox couple seems to help drive the ET behavior into the tunneling regime, suggesting that the Fc\textsuperscript{+1/0} sites act as kinetic potholes (traps) in the serial electron hopping process. The activation energy barriers are little changed by the presence of ferrocenated ligands and are primarily determined by the cluster core size.

Keywords: Electrochemistry, Nanotechnology, NMR
Application Code: Nanotechnology
Methodology Code: Electrochemistry
Dr. Boris Belousov reported an oscillation reaction of the redox change of Ce³⁺ and Ce⁴⁺, and Dr. Anatol Zabotinsky improved the reaction sequence which had been investigated as Belousov-Zhabotinsky (BZ) reaction. To date, BZ reaction has been investigated as self-oscillating system toward a key feature of space-time self-assembly of nonequilibrium systems. The first complete mechanism for BZ reaction is known as the Field-Koros-Noyes (FKN) mechanism.¹ Porphyrin plays an important role in biomolecular system such as hemoglobin and chlorophyll derivatives. We have developed a self-oscillating gel actuator in the wake of BZ reaction catalyzed by ferroin for the first time,² which causes autonomous mechanical oscillation without an external control in a completely closed solution. Because ruthenium tris (2, 2'-bipyridine) ruthenium only has been used as the conventional self-oscillating gel systems. In this light, BZ reaction system driven by water-soluble porphyrin derivatives was designed to clarify self-oscillating redox potential of porphyrin derivatives. This presentation is concerned with the self-oscillating reaction driven by water-soluble metallo-porphyrin derivatives for the first time. This system is of interest as a potential circadian rhythm mechanism and as a possible space-time self-assembly can be promoted by chemical reduction and oxidation system.

References

Keywords: Biotechnology, Nanotechnology, Rheology, Solution
Application Code: Nanotechnology
Methodology Code: Chemical Methods
Chemical Methods

Eliminate TKN and Obtain Better Total Nitrogen Data

Nitrogen is a principle element referred to when discussing nutrient pollution and, along with phosphorus, is largely responsible for the occurrence of algal blooms. The EPA approved method for Total Nitrogen, Total Kjeldahl Nitrogen (TKN) plus nitrate/nitrite, is cumbersome, time consuming, hazardous, and not very effective at determining total nitrogen in ambient water. Alternative methods are needed. This presentation evaluates two methods, a wet chemical oxidation that determines nitrogen colorimetrically and a high temperature oxidation method that determines nitrogen by chemiluminescence. Comparison data will be presented.

Keywords: Analysis, Environmental, Environmental Analysis, Environmental/Water
Application Code: Environmental
Methodology Code: Chemical Methods
Physico-Chemical Analysis of Drinking Water of Kheda District Gujarat, India

Physico-Chemical analysis such as temperature, pH, DO, Total hardness (TDS), Total alkalinity (TA), Calcium hardness (CaH), Magnesium hardness (MgH), chloride (Cl), sulphate (SO4)-2, Nitrate (NO3)- of water samples of twenty villages of Kheda district, Gujarat state, India. Quality of water is an important factor for drinking water is poor and quite good for drinking and irrigation purposes respectively. In the present communication deals with study of phosphates parameters is higher than the prescribed values. The higher values of phosphates are mainly due to the use of fertilizers and pesticides by the people residing in this area. If phosphate is consumed in excess phosphine gas is produced in gastrointestinal tract on reaction with gastric juice, similarly nitrate parameter is higher than the tolerance range. Nitrate, nitrogen is one of the major constituents of organism along with carbon and hydrogen as amino acids proteins and organic compounds in the drinking water. As there parameters of drinking water carried out during 2012-2013 in order to assess water quality index.

Keywords: Environmental/Water
Application Code: Environmental
Methodology Code: Chemical Methods
Ultra-thin silica shells (UTSS) are an effective way to improve the stability and compatibility of nanoparticles without significantly attenuating their intrinsic optical properties. This is of particular importance to plasmonic particles used in sensing or surface related studies such as localized surface plasmon resonance (LSPR) or surface enhanced Raman spectroscopy (SERS). For silver nanoparticles an UTSS can both hinder oxidation of the Ag in both air and water as well as establish a scaffold for functionalization through well-established silane chemistry.

UTSS on metallic nanoparticles such as gold or silver generally involve first using a silane coupling agent to render the surface vitreophilic followed by precipitation of silica by the Stober method or addition of sodium silicate species. Here we present a method to obtain silver nanoparticles with a silica shell of a thickness between 1-20 nm on silver nanoparticles ranging in size from 20-300 nm. It is obtained during the growth of the nanoparticles themselves by addition of a silica source to the well-established method of silver nanoparticle synthesis by hydrogen reduction of silver(I) oxide in water that both catalyzes the reaction as well as generates the shell. This allows for fairly monodisperse spherical silver nanoparticles to be obtained in concentrations of approximately 4.8E12 AgNP per litre (for 100 nm AgNP with 1 nm silica shell). This silica shell greatly improves the particle stability allowing for further concentration of the particles in water up to solutions containing 50% Ag by weight. In addition we demonstrated the ability of these particles to be easily integrated into substrates for both SERS and LSPR applications.

Keywords: Materials Characterization, Modified Silica, Particle Size and Distribution, Wet Chemical Methods
Application Code: Nanotechnology
Methodology Code: Chemical Methods
Representation of measurement capabilities is essential for the integration of manufacturing applications as well as management of instrument reliability. This study presents methodologies and results for an MCA study of trace impurities (CO, CO2, N2, H2, CH4, Ar, O2 and H2O) in Helium. Linearity of concentration and instrument response, static repeatability, accuracy, standard deviation, method detection limit (MDL), Gage Repeatability and Reproducibility (R& R) and stability are investigated. The majority of the testing performed within this study was conducted on VICI Trace Gas Analyzer (TGA) and Servomex O2 & Moisture dual analyzer at a Matheson facility. Finally, the instruments proved to be accurate, precise and reliable at a level sufficient to conduct the analysis of trace impurities in Helium.

Keywords: Quality, Trace Analysis
Application Code: Quality/QA/QC
Methodology Code: Chemical Methods
We describe a fast, efficient and mild approach to prepare chemically reduced graphene oxide (rGO) at room temperature. Divalent europium triflate \( \text{Eu(OTf)}^{2-} \) has been utilized as reducing agent for graphene oxide (GO). We have also characterized the solution-processable rGO by using various spectroscopic (FT-IR, UV-visible absorption and Raman), microscopic (TEM and AFM) and powder X-ray diffraction (XRD) techniques. A detailed kinetic analysis was performed which provides that, the bimolecular rate constants for the reduction of GO are \( 13.7 \pm 0.7 \, \text{M}^{-1} \text{s}^{-1} \) and \( 5.3 \pm 0.1 \, \text{M}^{-1} \text{s}^{-1} \) in tetrahydrofuran (THF)-water and acetonitrile (ACN)-water mixtures, respectively. The reduction rate constants are two orders of magnitude higher compared to the values obtained in the case of commonly used reducing agents such as hydrazine hydrate, sodium borohydride and glucose-ammonia mixture. The mechanistic details of the reaction were unfolded by determining the rate orders for various components in the reaction. The present work introduces a feasible reduction process for preparing graphene at ambient conditions, which is important for bulk production. More importantly, the study explores the possibilities of utilizing the unique chemistry of divalent lanthanide complexes for chemical modifications of GO.

**Keywords:** Characterization, Method Development, Spectroscopy, UV-VIS Absorbance/Luminescence

**Application Code:** General Interest

**Methodology Code:** UV/VIS
Carvacrol (5-isopropyl-2-methylphenol) and Thymol (2-isopropyl-5-methylphenol) the predominant monoterpenic phenol which occurs in many essential oils of the family Labiatae used through the ages as a source of flavor in food. Thymoquinone (2-isopropyl-5-methyl-1,4-benzoquinone) is an active ingredient isolated from Nigella sativa and has been investigated for its antioxidant, anti-inflammatory and anticancer activities. Moreover, thymoquinone could act as a free radical and superoxide radical scavenger, as well as preserving the activity of various anti-oxidant enzymes. Free radicals and other reactive oxygen and nitrogen species (ROS and RNS) are generated by all aerobic cells and are known to participate in a wide variety of biological and biochemical processes. H2O2 is a major ROS in living organisms, and its homeostasis can have diverse physiological and pathological consequences. In addition, H2O2 and Fe (II) can produce reactive •OH radicals by the Fenton reaction. Oxidants are connected to aging and severe human diseases such as cancer, cardiovascular disorders and related neurodegenerative diseases. In the present study, antioxidant effects of carvacrol, thymol and thymoquinone were investigated against the Fenton reaction. H2O2 and Fe (II) were added to each antioxidant solution. Oxidation of each antioxidant was monitored at time period by UV-visible spectrophotometer until the stabilization of reaction. Oxidative products and intact antioxidants were determined by HPLC-DAD. While Thymoquinone oxidizes to quinones, carvacrol and thymol also produces another antioxidant compound of thymoquinone.

References

Keywords: Bioanalytical, HPLC, Method Development, UV-VIS Absorbance/Luminescence
Application Code: Bioanalytical
Methodology Code: Chemical Methods
Oxygen concentration analysis relies on the conversion of sample oxygen to carbon monoxide following the temperature-dependent Boudouard equilibrium, which favors the formation of carbon monoxide over carbon dioxide with increasing temperature during pyrolysis. In the past, the available furnace technologies did not allow temperatures exceeding 1200 °C during pyrolysis. This was a limitation for highly precise oxygen concentration measurements, because full conversion of sample oxygen to carbon monoxide requires furnace temperatures in excess of 1400 °C. Using novel furnace technology based on a glassy carbon reaction tube inside a silicon carbide furnace, it is now possible to perform pyrolysis at a temperature of 1450 °C. Full conversion of sample oxygen to carbon monoxide, which is a prerequisite for highly precise and matrix-independent oxygen concentration analyses, is therefore possible. This improvement leads to oxygen concentration analyses with unprecedented precision (standard deviation <0.1 % absolute) and convenience. We present the applicability of the novel pyrolysis technique based on selected organic as well as inorganic samples.
It is often necessary to measure total organic carbon (TOC) <1 ng/mL in high purity water systems. This is especially true of the semiconductor industry where a statistical departure from baseline results in an abrupt system shutdown. Part per trillion TOC is most often measured by conductivity of dissolved CO2 produced upon complete oxidation of organics present. These systems are commonly batch type analyzers prone to interferences from various other conducting species produced during oxidation. We present a continuous method for measuring TOC without digestion. All compounds absorb in the deep UV which we measure using the 214 nm line from a zinc lamp and a fiber optic coupled CCD spectrometer. Cavity enhanced absorption spectroscopy is used to improve the sensitivity of the device by increasing the effective pathlength. The absorption cell was constructed of extruded polytetrafluoroethylene, a diffuse reflector highly reflective even in the UV. The physical pathlength was 25 cm, but an effective pathlength of up to 280 cm was achieved. The system was evaluated using Potassium hydrogen phthalate (KHP) and glucose. Limits of detections for KHP and glucose were 0.484 nM and 64 nM respectively, or 46.5 ng C/L and 4.6 mg C/L. These values represent likely upper and lower bounds of sensitivity with real samples falling somewhere in between. Such a device may prove useful in identifying potential sources of contamination when used in conjunction with other TOC analyzers.

This research was supported through a grant from the National Science Foundation; NSF CHE1246368.

Keywords: Monitoring, Total Organic Carbon, Ultratrace Analysis, UV-VIS Absorbance/Luminescence
Application Code: General Interest
Methodology Code: UV/VIS
Chemical Methods

A Study of Physico-Chemical and Fertility Analysis of Soil Samples of Rural Area of Kheda, Gujarat (India)

The soil samples from different fields of rural area surrounding Kheda, Gujarat (India) were collected. Some Physico-Chemical analysis such as Temp, pH & Conductivity was studied. Some fertility analysis such as Nitrogen(N), Phosphorus(P), Potash(K) was studied. The soil samples were collected from different fields located at KHEDA in colorless polyethylene bag with necessary precaution. The soil samples were carried out in May-2010. The analysis of samples were carried out during May-2010 To Aug-2010. The major soil problems in India cause various kinds of soil degradation are soil erosion loss of fertility, salinity and alkalinity, acidity water longing and deterioration of soil structures. The survey numbers of the fields were collected from the Dist. Agricultural Office, Kheda, Gujarat (India).

Keywords: Chemical, Soil
Application Code: Environmental
Methodology Code: Chemical Methods
Chemical Methods
Spectrochemical Characterizations of a New Water Soluble O-Carboxymethyl Chitosan Schiff Base and Its Pd(II) Complex

Chitin is the second abundant biopolymer after cellulose in nature. Chitosan is derived from deacetylation treatment of chitin in alkaline media. Its physicochemical and biological properties make it worthy in regard to many areas like medicine, cosmetic food, agriculture and biotechnology. However, its usage limited, because chitosan does not dissolve in water and in common organic solvents. To improve the solubility of chitosan and extending its usability many derivatives of chitosan have been synthesized and carboxymethyl chitosan (OCMCS) is an important derivative.

In the present study, for solubility problem of chitosan in aqueous media was overcome, a new water-soluble O-carboxymethyl chitosan Schiff base (OCMCS-3a) and its Pd(II) complexes was synthesized. Firstly, 3a (mono-imin) was synthesized via condensation reaction between 2,4 pentadion with 3-aminobenzoic acid in alcohol (1:1). The CS-3a was obtained as a result of reaction of free oxo groups of mono-imin (3a) and with amino groups on the chitosan. Then, water soluble forms of CS-3a was obtained through oxidation of hydroxide groups on the chitosan to the carboxymethyl groups with monochloracetic acid (OCMCS-3a) [1]. Pd(II) complex of OCMCS-3a was synthesized in water. Characterization of OCMCS and its Pd(II)complex was conducted using FTIR, 1H-NMR, 13C-NMR, UV-Vis, TG/DTG, ICP, XRD, and SEM techniques.

Acknowledgements
The authors thank The Scientific and Technological Research Council of Turkey (TUBITAK 113Z296) and Aksaray University Scientific Research Projects Coordination for financial supports (Project Number: 2012-20)

Reference

Keywords: Biological Samples, Characterization, Spectroscopy, Thermal Analysis
Application Code: Other
Methodology Code: Physical Measurements
Hydrogen cyanide (HCN) is a highly toxic, water soluble molecule that is readily absorbed by aquatic organisms through contact with skin and mucous membranes (e.g. gills) and transported into the bloodstream. Section 304 (a) of the Clean Water Act requires the U.S. Environmental Protection Agency to publish and periodically update ambient water quality criteria (AWQC). The criteria establish maximum threshold concentrations of contaminants for freshwater and marine environments based on empirical toxicity data. Concentrations of free HCN in surface waters can range from 0.005 to several mg HCN/L. U.S. EPA acute and chronic AWQC for free cyanide in freshwater are 22 $\mu$g CN/L and 5.2 $\mu$g CN/L respectively. Rainbow trout (Oncorhynchus mykiss) were found to be the most acutely sensitive freshwater species to cyanide.

In May of 2012 the U.S. EPA approved ASTM Method D 7237-10 for analysis of free cyanide in environmental and NPDES wastewater samples. ASTM D 7237-10 is a flow injection analysis method which employs the gas diffusion amperometry technique to avoid analytical interferences encountered with earlier methods.

This poster will present performance data on ASTM D 7237-10 and application of the method to measure free cyanide and cyanide bound in metal-cyanide complexes that dissociate into free cyanide ions at the pH of an aquatic environment (pH 6 to 8).

Keywords: Environmental/Water, Flow Injection Analysis, Water, Wet Chemical Methods

Application Code: Environmental

Methodology Code: Chemical Methods
Chemical Methods

Scavenging of Residual Impurities and Coupling Reactions

Traditional reaction's optimization is often cumbersome and time consuming. Significant efforts by manufacturers of automated systems have been directed at increasing the speed, efficiency, and consistency in performing chemical reactions. To date, the numbers of integrated systems that combine all the relevant steps of reactions preparations are few. The main feature of the SiliCycle® MiniBlock® is to offer a unique approach to support reaction's optimization and workup of diverse compounds without compromising reagent types or conditions used.

The SiliCycle MiniBlock has fritted reaction vessels, and combined with its patented built-in valve; solid-liquid phase reactions are made easy and elegant: tedious solid washing steps can be performed by simply opening/closing the valve in order to remove or add solvents. Vortex mixing ensures thorough agitation during the reactions.

Results for a number of fundamental reactions, including scavenging of residual impurities, coupling reactions, will be presented in this poster to demonstrate the versatility and the usefulness of the SiliCycle MiniBlock.

Abstract Text

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Keywords: Accelerated Solvent Extraction, Laboratory, Sample Handling/Automation, Sample Preparation
Application Code: General Interest
Methodology Code: Chemical Methods
Gold nanoshells consisting of a dielectric core with a gold outer layer are known to have absorption and light scattering properties that extend into the NIR region of the electromagnetic spectrum. This spectral feature enables gold nanoshelled particles to be used in biological and optical applications. Silica and polystyrene nanoparticles are commonly used as core materials with gold nanoshells. However, these materials have rigid physical properties which can limit their potential applications. Here, we use an approach involving nanomaterials with tunable properties. This new class of nanomaterials is derived from a group of uniform material based on organic salts (GUMBOS).

We have prepared thiol-functionalized GUMBOS, and prepared nanoGUMBOS by reprecipitation. Gold nanoshells are prepared by attaching Tetrakis(hydroxymethyl)phosphonium chloride (THPC)-gold seeds to thiol-functionalized nanoGUMBOS. The gold shells grow by mixing gold seeded nanoGUMBOS with gold hydroxide solution. Shell thickness is tuned by changing the ratio of gold hydroxide solution to gold-seeded nanoGUMBOS. Shifted UV-Vis absorbance spectra are observed according to the varied shell thickness.

This class of materials is versatile in that GUMBOS can be composed of an array of ionic combinations. More optical and biological properties will be studied with varied nanoGUMBOS cores.

Keywords: Nanotechnology, Near Infrared, UV-VIS Absorbance/Luminescence
Application Code: Nanotechnology
Methodology Code: Chemical Methods
As an important biological indicator of cancer status and progression, biomarkers represent powerful tools for cancer study. Cancer biomarkers are molecules secreted by tumor or a specific response of the patient body to the presence of cancer and could be used for cancer diagnosis and prognosis. Among them, non-invasive cancer biomarkers are promising due to the easy collection process. They all originate from tumoral tissue to blood, urine and other body fluids and many isolation and detection techniques have been established targeting these cancer biomarkers. Despite the intense interest in biomarker development for cancer management, few biomarker assays have been approved by FDA. Introduction of aptamer may offer a novel platform for cancer biomarker research, due to its unique properties and ability to work with many analysis technologies. The goal of this project is to develop new aptamer-based cancer diagnosis methods for microvesicle, a promising non-invasive cancer biomarkers.

The Main aim of this project is development of cancer microvesicle aptamer and its application in cancer diagnosis. Cancer microvesicles exist in several bioactive fluids and perform as one non-invasive biomarker. Characterization and aptamer development have been done. The specific binding between aptamer and microvesicle have been demonstrated. Furthermore, by introducing microvesicle aptamer to microfluidics and other methods, target microvesicle separation and other application in cancer diagnosis have been studied. This projects will demonstrate the feasibility of aptamer-based assays as effective platform to distinguish and isolate non-invasive cancer biomarker. In addition, these studies will eventually diverse and versatile applications in cancer diagnosis.

Keywords: Analysis, Bioanalytical, Clinical/Toxicology
Application Code: Bioanalytical
Methodology Code: Chemical Methods
Vanadium dioxide (VO2) is a unique transition metal oxide that undergoes a first-order phase transition at approximately 340K from the insulating monoclinic phase (M) to the conducting rutile phase (R). This metal-to-insulator transition (MIT) results in a change in the resistivity and optical absorption of the material, which has made it useful in several electronic and electro-optic applications. Common synthetic techniques such as pulsed laser deposition (PLD), chemical vapor deposition (CVD), and magnetron sputtering allow for the synthesis of high quality films of VO2 for these applications. Recently, however, hydrothermal methods have been used for the synthesis of relatively large quantities of VO2(M) from inexpensive starting materials without the need for expensive instrumentation. While these hydrothermally synthesized VO2(M) particles have thus far been unsuitable for electronic and electro-optic applications, their applicability in other areas, such as catalysis and sensing, is still being evaluated.

In this work, the amount of vanadium precursor present during a hydrothermal treatment was varied to produce several different morphologies of VO2(M) including plates, snowflake asterisks, and truncated asterisks. Films of the particles were drop cast onto glass slides and four-point Van der Pauw resistivity measurements were used to determine the phase transition behavior of the particle films. We report, for the first time, resistivity data on drop cast films of VO2(M) with resistivity changes of up to two orders of magnitude. Additionally, BET gas adsorption theory was used to determine the surface area of the particles in order to evaluate their potential as catalytic materials.

Keywords: Materials Science, Method Development, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Chemical Methods
Chemical Methods
Automated, In-Line Extraction and Analysis of Methylene Blue Active Substances (MBAS) in Waters

Manual, colorimetric chemistries have been a mainstay in the lab for many decades. Many chemistries require complex sample preparation steps, prior to the analysis. The combination of budgetary constraints and increased sample loads is making automated chemistries not only a nice to have, but an absolute must.

Since 2007, the USEPA has begun to allow automation of sample preparation steps as part of CWA (Clean Water Act) methods. Flow Injection Analysis (FIA) is one automated technique that allows automation of sample preparation steps, in addition to the automation of the colorimetric chemistry.

The manual determination of surfactants, (MBAS) includes an extremely complex, time-consuming and error prone extraction method which also involves extended exposures to chloroform for the analyst. Two Lachat methods will be shown, that automate both the extraction and the colorimetric determination. These methods also maintains the chloroform in a closed system to minimize exposure to the operator.

The methods utilize an in-line, dual extraction, whereby interferences are reduced by either a second extraction in an alkaline solution of methylene blue or a second wash with phosphate buffer (equivalent to SM 5540-C/ASTM D2330-02). These methods use a single membrane for phase separation, have rapid start-up and shut down times, and significantly reduce the amount of chloroform needed for each sample. 24 samples/standards per hour can be analyzed using these methods.

Keywords: Automation, Environmental/Water
Application Code: Environmental
Methodology Code: Chemical Methods
Stable and monodispersed silver (~2-50nm) and gold nanoparticles (~40 nm in size) were produced through a one-pot synthetic approach utilizing the reduction of silver nitrate and gold (iii) iodide respectively in the presence of polyamic acid (PAA) as a reductant and stabilizer. The advantages of PAA as stabilizer are not only the enhancement of long-term stability, adjustment of the solubility and amphiphilicity of nanoparticles, but also the functionalization of nanoparticles with its amide and carboxylic moieties to achieve higher and tunable surface-density of shell/brush as well as to promote compatibility and processability. Stabilization using the PAA is by physisorption and metal nanoparticles are sterically and/or electrostatically stabilized against aggregation by the oxidized polymer. TEM, HRTEM, EDS, SAED and UV-VIS were used to characterize the nanoparticles. The effect of temperature on the formation of silver nanoparticles was also studied.


Keywords: Characterization, Materials Characterization, UV-VIS Absorbance/Luminescence
Application Code: Nanotechnology
Methodology Code: Chemical Methods
Current explosives trace detectors (ETD) require human operations for swab-sampling of contaminants from surface of baggage. The throughput of commercial explosives trace detection portals, lower than 500 people per hour, is not high enough for investigations at ticket gates of train stations and airports, where more than 1000 people per hour pass through.

To analyze less-volatile explosives such as TNT, PETN, and RDX, we developed a novel particle sampler using a cyclone-type particle concentrator, which can collect and concentrate the explosive particles attached on the surface effectively and rapidly [1]. The explosives particles collected by the sampler are then vaporized by a heating unit that is located below the concentrator, introduced into a mass spectrometer.

We tested a prototype using NIST SRM2905, trace particulate explosive simulants. The ng-level of the particle explosive simulants, which included ng-level of TNT, were adhered to IC-cards ticket and we observed TNT signal within 1-2 seconds after the emission of the air jet from the nozzle to IC-cards ticket. We measured the false-positive rate with over 850 volunteer passengers at a train station in Japan to evaluate the false-positive rate. ROC (Receiver Operating Characteristic) curves were created from the field test data for both single-marker and multiple-marker analysis. The field test data showed that the multiple-marker analysis was more selective than single-marker analysis, which achieved a low false-positive rate of about 0.1%.

This work was supported by "R&D Program for Implementation of Anti-Crime and Anti-Terrorism Technologies for a Safe and Secure Society”, Strategic Funds for the Promotion of Science and Technology of the Ministry of Education, Culture, Sports, Science and Technology, Japan.


Keywords: Detection, Mass Spectrometry, Trace Analysis
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
The goal of this research is to extend previous work on a spatial heterodyne Raman spectrometer, SHRS, by investigating the issues that affect the sensitivity of SHRS for standoff detection of minimally or non-absorbing solutions. In the SHRS, the high optical throughput of the interferometer offers high sensitivity, however this may not be realized in standoff applications where the etendue is limited by the telescope used for light collection. Unlike the case of a tightly focused laser beam, the throughput advantages of the SHRS may be realized for larger laser spots on the sample, where the SHRS can provide higher light throughout because of its wide field of view. Large laser spots on the sample may be useful for minimizing laser-induced damage or for quickly characterizing a large sample, with a secondary benefit of minimizing the effect of laser pointing stability on a remotely-located sample. In this paper, we will present standoff SHRS Raman spectra for a series of solution samples including nitrates, perchlorates, sulfates, and explosive, such as TNT and RDX. The effect of system parameters such as laser spot size, optical filtering, and coupling optics on sensitivity and signal-to-noise for samples that show both simple and complex Raman spectra will be discussed, as well as samples that fluoresce. Throughput trade-offs when using a telescope for standoff Raman measurements will also be discussed.

**Keywords:** Laser, Raman, Solution, Spectrometer

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Molecular Spectroscopy
Sensitive infrastructures such as e.g. airports have to be monitored to prevent fire disasters or protected against potential attacks using hazardous compounds such as chemical warfare agents (CWA) and toxic industrial chemicals (TIC). Several accidents e.g. at the Düsseldorf airport in 1996 with 17 killed people and a damage of 500Mio€ show the great potential of destruction. On the other hand airports a subject to terrorist attacks e.g. at the Moscow airport in 2011 with 35 killed people. It is advantageous to have a supervision system that can monitor both cases.

In this paper we will present a stationary detection system based on ion mobility spectrometer (IMS) in combination with other orthogonal sensors like photo ionization detector (PID), electrochemical cells (EC) and metal oxide sensors (MOS). In the new developed IMS we are using a non radioactive ion source. With that a lot of national and international regulation regarding radioactive sources can be neglected. The various chemical gas sensing principles operated simultaneously provide a broad detection range with very short response times. The combination enhances the selectivity of the system in order to achieve low false alarm rates. Cross sensitivity is reduced by using the increased information content in the detectors response. The stationary detection system is designed for 24/7/365 operation. Beside open fires and unknown sources, fires cause by defect electrical systems on the third place for causing disastrous fires. It is desirable to detect fires before main outbreak in a very early stage, already as smoldering fire.

In this paper we will present measurement showing the ability of the stationary detection system to detect fire already in a very early stage of smoldering fire. We will present and discuss the advantages of a hybrid sensor array for multipurpose detection tasks and the versatile application for safety and security monitoring usage.

Keywords: Array Detectors, Contamination, Detection, Identification
Application Code: Homeland Security/Forensics
Methodology Code: Integrated Sensor Systems
Polycyclic Aromatic Hydrocarbons (PAHs) are a large group of organic compounds found naturally in the environment. PAHs are monitored by the US Environmental Protection Agency due to their carcinogenic characteristics. In recent global events, PAHs have come to the attention for contamination in sea food samples due to the oil spills. In the oceanic environment, PAHs are bio-available via the food chain and contaminated sediments which could have the potential to bioaccumulate in the marine organism we eat.

QuEChERS is a Quick-Easy-Cheap-Effective-Rugged-Safe extraction method that has been developed for the determination of pesticide residues in agricultural commodities. Since its installment in 2003, QuEChERS has been adapted for use with many additional matrices and compound classes such as sea food and PAHs.

The aim of this project is to validate the extraction performance of the AutoMate-Q40 by monitoring Polycyclic Aromatic Hydrocarbons (PAH) extracted from sea food. The target compounds were determined by gas chromatography mass spectrometry (GC-MS).

Keywords: Food Contaminants, Gas Chromatography/Mass Spectrometry, PAH, Sample Handling/Automation
Application Code: Food Contaminants
Methodology Code: Gas Chromatography/Mass Spectrometry
Food Contaminants

A Simple and Rapid Extraction Method of Rhodamine B Detection in Raw Samples Using SERS

Rhodamine B has been abused in food industry in coloring and brightening numerous food products due to its bright color, high dyeing efficiency and low cost. In response, many analytical and physical chemical methods have been developed such as GC-Mass, HPLC, Uv-vis to detect Rhodamine B in food samples. However, most of them are lab-based methods and time and labor consuming. SERS-based method has been developed in recent years and is an attractive method due to its high sensitivity, intrinsic selectivity and nondestructive data acquisition and without accompanying with extraction method restricts its applications. Here we introduce a SERS-based detection method (SHINERS) using 55nm AuNPs as Raman signaling amplifying substrate and a novel liquid-liquid extraction route to detect Rhodamine B from raw fruit. The whole detection process can be completed within 10 min through a few simple steps and is able to achieve a detection limit down to 0.2ppm Rhodamine B concentration. More significantly, the result demonstrates no noticeable false positive and false negative with satisfied consistency, which extends the SERS detection method to in situ examination of suspicious food products. We have determined Rhodamine B with a simple extraction method and most common engineering AuNPs ,which can be developed into a successful commercial method.

Keywords: Food Contaminants, Food Identification, Food Safety, Raman

Application Code: Food Contaminants
Methodology Code: Process Analytical Techniques
Entering the composition of many processed foods as a base ingredient, the wheat must be of high and constant quality. The presence of microbiologically tainted wheat can indeed contaminate large batches of other raw material in storage or even prevail into the finished product if not removed from the process. The evaluation of wheat contamination is commonly carried out by visual assessment and human smelling at reception of raw materials. This study proposes to evaluate wheat and detect possible off-flavors with HERACLES electronic nose, based on ultra fast chromatography. Eight samples of wheat were analysed, four of good quality and four considered as tainted. The comparison of chromatograms obtained with HERACLES e-nose shows significant differences in terms of VOCs concentration between good and bad batches of wheat. Several volatile compounds (mainly alcohols and geosmin) have been detected at much higher concentration in tainted wheat than in good samples. Upon building an odor map based on the global aroma profile, the discrimination between good and bad wheats is clear. Also this Principal Components Analysis confirms that the differentiation is mainly linked with alcohols concentration. These compounds are thus responsible for the off-flavors. The electronic nose can thus be successfully used to rapidly assess and monitor the sensory quality of wheat.

Keywords: Food Contaminants, Food Science, Gas Chromatography, Quality Control
Application Code: Food Contaminants
Methodology Code: Gas Chromatography
Pressure sensitive labels are often pasted onto stretchable plastic food wraps containing meat, dairy products, and other food commodities. They can also be applied directly onto many produce items such as fruits and vegetables. In spite of such diverse usage, the US FDA possesses little or no relevant migration data on components of pressure sensitive adhesives. The majority of modern pressure sensitive adhesives are prepared from the following compounds: vinyl acetate, ethylene, di-2-ethylhexyl maleate (DEHM) and/or fumarate (DEHF), 2-ethylhexyl acrylate and carboxylic acid. In this work, GC-MSD has been used for the determination of the migration of DEHM and DEHF from adhesive labels to two solvents, 50% ethanol and isooctane, which are can be used as food simulants for lower and high fat foods.

The initial migration studies were performed at 40°C for 10 days and indicate that the highest combined amounts of DEHM and DEHF migrating from adhesive labels through a typical low-density polyethylene (LDPE) wrap is 18 ng/cm² for 50% ethanol and 69 ng/cm² for isooctane. Without the LDPE wrap, the highest amounts of DEHM and DEHF into 50% ethanol and isooctane are 50 and 8,000 ng/cm², respectively.

Because 50% ethanol is generally considered to be an acceptable food simulant for meat products, this migration data would provide fair estimates for the migration of DEHM and DEHF into meat products through an LDPE film. Since fruits and vegetables generally have low fat concentrations, data from the 50% ethanol migration study should conservatively estimate migration. This establishes that the expected combined migration of DEHM and DEHF is not expected exceed 50 ng/cm² in meat, dairy, and produce through a LDPE film. In addition, due to low consumption factors for products using these adhesive labels, consumer exposure from adhesive components from pressure sensitive labels is expected to be below the threshold of regulation.

Keywords: Food Contaminants, GC-MS
Application Code: Food Contaminants
Methodology Code: Gas Chromatography/Mass Spectrometry
While pea protein is a good source of vegetarian protein for nutritional products, it could trigger allergic reaction for some consumers. The analytical requirement to quantitate pea protein from trace amount to nutritional relevant amount remains a challenging problem. Conventional methods like Kjeldahl or Dumas measuring total Nitrogen without any specificity to different protein sources or sensitivity for trace allergen analysis. Other technologies like ELISA and HPLC also face the problems associated with complex matrix and protein denaturation from the process. We propose a novel approach for pea protein quantification by indirectly measuring genetic material deoxyribonucleic acid (DNA) present in the protein commodity and correlating the DNA quantity to protein quantity. Real-time polymerase chain reaction (qPCR) coupled with fluorescent probe for DNA quantification offers high selectivity of pea species from the primer design, high sensitivity from the qPCR technology, and broad quantification range. In our lab, we developed the method for quantitating pea protein in finished product of formulated milk powder, with linear range from 16.2 ppm to 16200 ppm with a regression coefficient (R2) of 0.9987. The whole workflow from sample purification to detection was optimized to achieve PCR efficiency of 103% and less than 4 hours total run time. This newly developed method could be utilized to quantitate pea protein from trace level to a nutritional normal level in a complex formulated milk product.

Keywords: Bioanalytical, Food Contaminants, Food Identification, Protein
Application Code: Food Contaminants
Methodology Code: Fluorescence/Luminescence
The identification of pesticide residues in foodstuffs by low resolution GC or LC-MS, commonly used in the monitoring of pesticides in this products, is frequently affected by high false positive (FP) and false negative (FN) rates due to instrumental limitations and the inadequacy of identification criteria [1]. This situation is a consequence of the difficulty to estimate the identification quality. This work presents a strategy for the selection of characteristic GC-MS signals of the analyte, the definition of adequate statistical criterion for the identification parameters and the reliable quantification of the uncertainty associated with analyte identity. The identification of compounds is based in the retention time and two ratios of the abundance of characteristic fragments of their mass spectra. Adequate probabilistic models were developed to handle the correlation between the abundance of fragments and of the non-normality of their ratio. The uncertainty of the identification is determined using Bayesian metrics [2] due to their ability to combine, in one parameter, the information from FP and FN. The FN was estimated by probabilistic models of the signal of the analyte and the FP by modelling the variability of signals of matrices free from pesticides. This tool was successfully used to estimate the reduction of the probability of identifications being correct as analyte concentration decreases and sample matrix complexity increases.

This research is financed by Fundação para a Ciência e a Tecnologia (EXPL/QEQ-QAN/0458/2013)

Biogenic amines are biologically active nitrogenous compounds of low molecular weight and are formed through decarboxylation reactions of amino acids. The presence of biogenic amines in food is a strong indicator of food spoilage, which can lead to food poisoning when consumed. Previous work utilized the activated sulfonate or carboxylic ester of various dyes coupled to hydroxybenzotriazole (HOBt). This activated dye-HOBt bond was susceptible to cleavage from water (i.e. hydrolysis). Recent advancements in reagent design have produced a dye-coupled HOBt that is resistant to hydrolysis.

The goal of this research is to utilize this product in a colorimetric assay for the detection of aqueous biogenic amines. Specific targets are those biogenic amines commonly found in spoiled food products such as histamine, cadaverine, and putrescine. For this research, isopropylamine (a primary amine) is utilized as a less hazardous analog for the target analytes. It has already been shown that methyl red (MR) is visible to the naked eye in concentrations as low as 500 nM, and that the new HOBt-coupled dye (HOBt-MR) resists hydrolysis for over 48 hours.

In this presentation, optimization of reaction conditions for the HOBt-catalyzed detection of amines will be discussed in detail. Specifically, the optimized conditions for temperature, time, ethanol content and buffer strength are presented. Future work includes the development of a new colorimetric assay for the quantification of total amines in fish flesh. This assay would allow more accurate determination of spoilage and exhibit no interference from water.
Food Contaminants

Development of a Sensitive Headspace-Gas Chromatography Mass Spectrometry Method for Off-Flavor Compounds in Water

Off-flavor compounds are described as water-borne, organic chemicals that are deposited into surface water sources causing undesirable tastes and odors. These compounds have been recognized as a worldwide concern since the late 19th century, particularly affecting surface water sources such as streams, rivers, lakes, wetlands, and oceans. Additionally, attention has been increasing toward the effects off-flavors have in aquaculture. The following compounds, isopropyl methoxypyrazine (IPMP), isobutyl methoxypyrazine (IBMP), methylisoborneol (MIB), and geosmin, potentially produced in aquaculture systems cause musty and earthy odors in fish resulting in decreased quality of the crop. The overall goal for this research is to develop and validate an analytical method for the quantitation of specific off-flavor compounds at lower limits of detection than previously studied. Traditionally, vigorous, time-consuming, and expensive pre-concentration steps were required to detect the compounds. This study has implemented a unique pressure-balance headspace sampling system coupled to a gas chromatography mass spectrometer. The headspace technique eliminates the need for organic solvents, reduces sample carryover, and incorporates trapping capabilities for maximum extraction. Resulting data has simultaneously separated and detected all four analytes of interest in less than 7 minutes. Also, further method optimization of trapping parameters was configured to achieve limits of detection below 1 ppt. The proper identification for the analytes of interest will allow future profiling of the compounds amongst aquaculture systems for possible correlation to a definitive source.

Keywords: GC-MS
Application Code: Food Contaminants
Methodology Code: Gas Chromatography/Mass Spectrometry
Many important decisions are based on results from instrumental methods of analysis such as the price of goods, the compliance of products with legal requirements or the outcome of research based on chemical analysis. A measurement procedure can only play its role properly if the reported information is objective and has the required quality. The reporting of measurement with uncertainty makes information objective since it defines an interval that should encompass the expected value of the measured quantity with a known probability. Different strategies for the evaluation of the measurement uncertainty can be used. The “bottom-up” approach involves dissecting the measurement procedure in individual uncertainty components responsible either for random or systematic effects, the quantification of these components and the determination of the combined effect of the various components. This approach is difficult to apply due to the complexity of involved models.

This work consists in the development of tutorials for the validation of quantifications performed by instrumental methods of analysis based on least-squares regressions. These tutorials include: 1) the definition of the Limit of Detection and calibration range, 2) the definition of the procedure for calibrators preparation, 3) the evaluation of the applicability of the regression model for describing the calibration curve and 4) the assessment of the measurement uncertainty using the “bottom-up” approach. The various stages are completed in a sequence of user-friendly MS-Excel spreadsheets. These tools were developed to be used by less experienced analysts and to ensure the production of sound metrological information.
Fruits and plants contain a wide range of antioxidant compounds in tissues which are responsible for their antioxidant capacity. These antioxidants include vitamins, carotenoids, phenolic acids and flavonoids which could play an important role since they behave as free radical scavengers, singlet and triplet oxygen quenchers and metal-chelators [1]. Several methods have been reported for determination of antioxidant capacity in plants and foods. Antioxidant capacity methods differ in terms of their assay principle and experimental conditions. Because of multiple reaction characteristics and mechanisms, a single assay will not accurately reflect all antioxidant in a mixed or complex system [2].

The CHROMAC method was used for determination antioxidant capacities of individual and combined phenolic compounds. The CHROMAC is a novel spectrophotometric total antioxidant capacity assay based on the reaction of excessive Cr(VI) and phenolic compounds and formation of coloured complex with remaining Cr(VI) and diphenylcarbazide [3]. The effects of pH, Cr(VI) and diphenylcarbazide concentration on the CHROMAC method were investigated. The reaction mechanism was controlled with the presence of chromium species of Cr(III), Cr(V),Cr(IV) and dipheylcarbazone in the reaction medium. The oxidation percentage of phenolic compounds by Cr(VI) were determined by chromatographic method. The antioxidant capacity determined by CHROMAC method was compared with chromatographic method.

The additivity of different classes of phenolic compounds was above 90% which implies that CHROMAC method is not influenced by other phenolics and interfering compounds. The TEAC values of different phenolic compounds were calculated with CHROMAC method.

References
**Abstract Text**

Tobacco is a crop that is associated with a large variety of molds, insects, and viruses from seedbed to storage in warehouses after manufacturing. Growers and manufacturers will use various chemical agents attempting to control these problems known as plant protective products (PPPs). There is a wide range of pesticides used on tobacco crops as plant protective products. The issue with pesticides used on tobacco leaf is related to the leaf after harvest and processing into is manufacturing forms. Pyrolysis pesticide residue is thus inhaled by the smoker and as an environmental tobacco smoke which may be one source of pesticides in the body. Due to the use of more polar pesticides, LC-MSMS technique has been implemented by laboratories. Improvements in the QuEChERS method allow for a quick and efficient sample preparation approach for the analysis of pesticides from tobacco with LC-MSMS analysis.
Bisphenol A (BPA) is used in the manufacture of many plastics and epoxy resins that are used to make milk containers and liners to seal metal food containers. Over time some of the free BPA will be leaching out from the plastics and resins to the food it contacts. Because of BPA’s potential to act as an endocrine disrupter at low concentrations in humans, regulations have been enacted regarding BPA concentrations in food and beverages sold for human consumption, and associated methods for the detection and quantification of BPA in food and beverages have been developed.

This work presents a method for detection of low levels of BPA in milk and canned broths. Quantifying low levels of BPA in complex sample matrices, such as food samples, can present sample preparation challenges. Molecularly Imprinted Polymer (MIP) Solid Phase Extraction (SPE) is highly selective and considered the top choice for sample cleanup of a single compound or a class of structurally related compounds from difficult matrices. This method uses a BPA MIP SPE to extract and pre-concentrate BPA. Analysis was performed using LC with fluorescence detection. Recovery of BPA from milk at 1 ng/mL was over 80%, and from chicken broth spiked at 60 ng/mL was over 70% with good reproducibility. An unfortified beef broth was also analyzed and found to contain BPA at level greater than 60 ng/mL. We will discuss these results and address some practical issues related to the analyses.

Keywords: Food Contaminants, HPLC, Solid Phase Extraction
Application Code: Food Contaminants
Methodology Code: Sampling and Sample Preparation
Iodine is an essential element required for the synthesis of the thyroid hormones thyroxine (T4) and triiodothyronine (T3). These hormones play an important role in regulating cellular activity, growth, and brain development. For humans, dietary deficiencies and excessive iodine intake have been shown to lead to a number of developmental abnormalities, mental retardation, and goiter. A recommended dietary allowance (RDA) of 150 [micro]g/day and a tolerable upper intake level (UL) of 1,100 [micro]g/day for iodine has been established for adults in the U.S. For dogs, a RDA of 29.6 [micro]g/kg body weight has been established for iodine. However, no UL is currently available for dogs. Additionally, the thyroid hormones T4 and T3 are orally active and the consumption of gullet contaminated foods can cause thyrotoxicosis, a type of hyperthyroidism. Several incidences of thyrotoxicosis in humans in the U.S. lead to a ban on the use of gullet trimmings in foods like hamburger or sausage.

In this work a method was developed for the detection of iodide, iodate, thyroxine, and triiodothyronine in dog jerky treats by liquid chromatography coupled to inductively coupled plasma mass spectrometry (LC-ICP-MS). This method was used for the speciation of iodine in multiple dog jerky treats available for sale in the U.S. Total iodine analysis using a separate method was performed and a mass balance comparison was made with the sum of the species obtained from this work. Additionally, several dog jerky treats were fortified at multiple fortification levels for all of the analytes in this method.

Keywords: Elemental Analysis, Elemental Mass Spec, Food Contaminants, Liquid Chromatography
Application Code: Food Contaminants
Methodology Code: Liquid Chromatography/Mass Spectrometry
A significant number of cases of food adulteration have been in the global news recently. The economic adulteration of high value food products with lower cost, lower quality materials has a significant negative effect on the food industry and consumer confidence. In some cases, such as the adulteration of powdered infant milk with melamine, the consequences can be fatal.

Mid- and near-infrared spectroscopies are powerful analytical tools for detecting adulteration of food ingredients at economically relevant levels. A single rapid measurement can confirm material identification and quantitative property prediction as well as screening for contamination by undesired materials. Mid-infrared analysis is performed using a diamond ATR sampling accessory. Near-infrared analysis is performed using a near-infrared reflectance accessory (NIRA). Both of these sampling techniques provide fast results and can be used with no sample preparation.

A number of examples of mid- and near-infrared detection of adulterants in solid and liquid food products will be presented, demonstrating targeted and non-targeted methods of analysis. Qualitative and quantitative data is presented for each application as well as results from a novel algorithm for adulterants, capable of estimating the amount of each adulterant present and its detection limit. Method development and simple end-user deployment will be described as well as highlighting the advantages and disadvantages of the different statistical approaches to the analysis.
Pathogen contamination can lead to a variety of serious illnesses, and are often the reason behind recalls of consumer products. Current methods of bacterial detection are expensive and time consuming. As a result of the limitations of these methods, early detection of bacteria is difficult. Contaminated products may pass through the screening process undetected. In addition, these techniques are unable to determine whether or not any bacteria present are viable. If these techniques lead to the false conclusion that a food item is contaminated, an unnecessary recall of the item can ultimately have a negative impact on the economy, as well as leading to illnesses that may be severe. Electrochemistry use for the detection of bacteria has recently been implemented, and makes an ideal detection system due to the sensitivity, low cost, ability for miniaturization, and the rapidity of detection. Bacterial growth may also be monitored in real time, making electrochemistry an ideal method for early detection of pathogens. An autonomous electrochemical sensor was combined with a powerful multi-class Probabilistic Neural Network (PNN) system to classify four species of organisms (E. Coli #25922, E. Coli # 11775, S. Epidermis #12228, or C. Albicans #10231). This presentation will focus on the design, redox identification and classification of E.coli.
Natural vs. Synthetic Wine Corks: The Great Cork Debate from a Mercury Content Perspective

Much debate has been made about which type of cork is better for your wine: Natural or Synthetic? While that debate may never end, after our research, there will be no question about which type of cork has the most mercury content.

Mercury may or may not play a deciding role in the taste of your aged wine or bourbon, or even if your treasured bottle of wine ends up smelling like wet cardboard due to cork taint. However, mercury is a known toxic pollutant to humans and other animals that attacks the central nervous system, resulting in serious health problems. Mercury is everywhere in our environment, occurring naturally in fossil fuels, such as coal, crude oil, & natural gas, and it’s in our air, oceans, and freshwater sources greatly due to man’s industrialization of Earth. With the wide-spread deposition of mercury in our environment, it should come as no surprise that natural cork used for bottling of wine and other liquors may contain significant levels of mercury.

In this presentation, mercury concentrations in a variety of natural and synthetic corks measured using the Model MA-3000 Mercury Analyzer from Nippon Instruments Corporation (NIC) will be discussed in detail.

Keywords: Atomic Absorption, Environmental Analysis, Food Contaminants, Mercury
Application Code: Food Contaminants
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The purpose of this work was to assess specific migration (SM) of cadmium (Cd), lead (Pb) and chromium (Cr) in ceramic houseware into 4% acetic acid solution in accordance to ANVISA, the Brazilian Health Surveillance Agency. Twenty commercial samples of ceramic housewares, marketed in Campinas - São Paulo (Brazil), were studied. The samples were in contact with the 4% (m/v) acetic acid solution at 80 ºC for 2 hours and the technique of Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES) was applied for the quantification of the elements. The results showed that the samples analyzed presented values below the quantification limits of the method (LOQ) of Cd, Pb and Cr, corresponding to 1.80 [micro]g kg-1, 18.6 [micro]g kg -1 and 1.9 [micro]g kg-1, respectively. Therefore was observed that the specific migration of Cd and Pb into 4% (m/v) acetic acid solution, abide by the limits established by National Agency of Sanitary Surveillance (ANVISA), corresponding to 0.30 mg kg-1 and 4.0 mg kg -1, respectively. In Brazil does not have limit established for chromium.

Keywords: Food Contaminants, ICP, Metals
Application Code: Food Contaminants
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The adverse health risk of arsenic (As) and its widespread occurrence in the environment as well as in the food chain has raised great public concerns worldwide. Therefore, a routine screening of As concentrations in food materials has become more of an urgent task for food authorities in many countries. It is already well known that speciation, rather than just total As concentration is the key to fully understand the toxicity of As in food products.

HG-AFS offers several advantages for this task such as low cost, high sensitivity and fast analysis. Selective hydride generation atomic fluorescence spectrometry (SHG-AFS) has already been successfully applied to the analysis of inorganic and total arsenic in rice samples with method detection limits (MDL) of 1 and 3 ng g⁻¹, respectively. High performance liquid chromatography (HPLC) together with HG-AFS has also been used to provide the most precise speciation information in food material.

In this presentation, an automatic online SPE approach is demonstrated for arsenic speciation in food materials. The system is consisting of an autosampler, a 6-port injection valve and a HG-AFS analyser. Sample loop is made of an inline cartridge which contains strong anion exchange packing material. No HPLC was required. The loading of sample extracts, clean-up of the cartridge, elution and detection of the As species was done automatically using a software package developed in house. The performance of the system will be discussed in detail in the presentation.

Keywords: Atomic Spectroscopy, Food Contaminants, Hydride, Speciation
Application Code: Food Contaminants
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Triacylglycerides (TAGs), which form the bulk of vegetable and animal oils, are subject to polymerization reactions when heated. These reactions occur by thermal polymerization or by oxidative polymerization reaction via radicals. These polymeric compounds create high-boiling point, higher viscosity, and insoluble materials, which can affect product quality and properties. For cooking, these polymerized triglycerides (PTGs) can result in increased oil absorption into cooked foods and possibly unhealthful properties (reduced digestibility).

The current method for the determination of these PTGs is by high performance liquid chromatography (HPLC) with size exclusion chromatography (SEC) and refractive index (RI) detection, which also requires a solid phase extraction (SPE) process to remove these polymers from the TAGs. A reverse phase, gradient HPLC method was developed, using a C18 column and the Thermo Scientific™ Dionex™ Corona™ Veo™ charged aerosol detector. This method has several advantages: no SPE is required, as the TAGs will not interfere with the analysis of the PTGs, the chromatography provides more information in the equivalent time, and the detector is highly sensitive and precise, thereby improving data quality.

Charged aerosol detection (CAD) is a mass sensitive technique for determining levels of any non-volatile and many semi-volatile analytes after separation by HPLC. The use of CAD for the analysis of lipids is well documented, as it will detect any non-volatile analyte with a uniform response factor, and allows use of gradient methods. Unlike methods using evaporative light scattering (ELS) detection, HPLC methods using CAD have limits of detection of between mid-picograms to low nanograms on column and have a wide dynamic range from nanogram to microgram levels, with high reproducibility.

Comparative data from the results of this analysis with values in the literature is presented.
Tea flavors can vary from spicy to flowery to fruity and any combination thereof. Moreover, the flavor profile of tea can depend on where the tea leaves are grown, the brewing time and temperature, the processing of the tea leaves, and the type of leaf used. Using Head Space Solid Phase Micro Extraction (HS-SPME) sampling in conjunction with Gas Chromatography/Mass Spectrometry (GC/MS) for separation and analysis, assorted teas will be examined for their varied flavor components.

Keywords: Food Identification, Gas Chromatography/Mass Spectrometry, SPME
Application Code: Food Identification
Methodology Code: Sampling and Sample Preparation
Food Identification

The Qualitative and Quantitative Analysis of Steviol Glycosides by HPLC-PDA in Energy/Vitamin Drinks

In response to the increased focus on lowering sugar consumption from foods and beverages, food/beverage manufacturers are now introducing the highly touted sugar substitute rebaudioside A (Reb A), in place of all or most of the sugar in certain foods and beverages. The primary interest in Reb A is the fact that it is a naturally derived sweetener considered to be at least 400 times sweeter than sugar and, therefore, can be added to products in considerably lower concentrations. The use of Reb A has been especially accelerated now that it is considered as [i]Generally Recognized as Safe[/i] (GRAS) by the U.S. FDA.\(^1\)

Reb A and stevioside, both steviol glycosides, are the primary extracts from the Stevia rebaudiana plant from South America, particularly Paraguay. As the use of stevia extracts as sweeteners has gained significant momentum, due to its limited availability, there is a growing concern of both adulteration and label claim accuracy in products reported to contain these extracts. With the above in mind, this application highlights the HPLC separation of Reb A, stevioside, Reb B and Reb C, as well as the analysis of these components in a selection of three energy/vitamin drinks. The chromatographic conditions were so chosen as to closely match the latest monograph covering steviol glycosides, including the four listed above, published by the Joint FAO/WHO Expert Commission on Food Additives (JECFA).\(^2\) This monograph, specifying the use of HPLC and a UV-based detector, is considered the internationally recognized method for analyzing steviol glycosides in food and beverage products.


Keywords: Beverage, Carbohydrates, Food Identification, HPLC
Application Code: Food Identification
Methodology Code: Liquid Chromatography
Verification of provenance is important for food authenticity. With high value foods, the country of origin has a dramatic effect on the price. Falsifying the provenance of foods is an attractive proposition to fraudsters. An example is olive oil, being marked incorrectly from desirable regions. Whilst various techniques such as trace metal analysis or isotopic ratios have been shown to separate different geographical classes, other factors such as crop variations and weather conditions have caused these separations to be inconclusive. The use of more than one technique to differentiate can be more convincing. This work uses TIBCO Spotfire® advanced analytics and visualization tools to separate the cultivars and origin of olive oils with a combination of different LC/MS results.

Samples of 35 different monovarietal and blended oils were analyzed using a PerkinElmer HPLC system, with reversed-phase gradients on a C18 column. Detection was with an accurate mass electrospray instrument, the PerkinElmer Axion TOF.

Levels of various classes of compounds in the oils were measured after different sample preparation methods. Triacylglycerides were analyzed from diluted oils in positive mode, the phenolics and free fatty acids from an aqueous extraction of the oils and analysis in both positive and negative modes. All datasets were processed with a unique algorithm to extract levels of compounds and results combined for statistical analysis. Data fusion allowed a better separation of samples into cultivars than any single analysis method. Some regional grouping of samples within a cultivar was also possible.

Keywords: Chemometrics, Food Science, Statistical Data Analysis
Application Code: Food Identification
Methodology Code: Chemometrics
Globally the popularity of nutraceutical products continues to increase, such natural product remedies are found in foods, roots and herbs. The legislative focus has resulted in a growth in method development to analyse active compounds in such products.

Korean ginseng, ginko biloba, red panax extracts and ginsenocide standards were analysed using ultra performance liquid chromatography (UPLC). A UPLC® HSS T3 (100mm x 2.1mm, 1.8[micro]m) analytical column was utilized. The chromatographic conditions consisted of a 20 minute water (0.1% Formic Acid)/acetonitrile (0.1% Formic Acid) gradient at 0.6 ml/min, using a sample injection volume of 1µl. Negative ion electrospray with ion mobility data acquisition was performed using a Synapt G2-S mass spectrometer.

Non targeted UPLC ion mobility mass spectrometry has been used to generate collision cross sections (CCS), precursor ion accurate mass, accurate mass fragment ions and retention times to profile ginsenocide standards Rb1, (Rb2, Rc), (Rd, Re), (Rf,Rg1) and Rg2. This data was utilised to create a scientific library incorporating the expected CCS values. Three extracts, ginko biloba, red panax and korean ginseng were analysed and routinely screened against the created ginsenoside CCS library, to determine the presence/unequivocal identification of the ginsenoside isomers. When comparing the expected against the measured collision cross sections determined (for the all eight ginseocides profiled in the extracts), the CCS measurement errors were typically <0.5%.

This approach offers a unique selectivity in profiling complex mixtures. The results obtained clearly show the benefits of using the collision cross section measurements and the combined peak capacity of UPLC and ion mobility. Co-eluting analytes and isomers have been resolved as well as unequivocally identified in the three extracts profiled. The approach changes the scope of authentication profiling.

Keywords: Characterization, Food Identification, Instrumentation, Liquid Chromatography/Mass Spectroscopy

Application Code: Food Identification

Methodology Code: Liquid Chromatography/Mass Spectrometry
### Abstract Text

Milk proteins concentrates and isolates are used as an ingredient in a wide variety of food applications, they are commonly found in infant formulas, enteral nutrition, bakery products, desserts, meat products, dairy based dry mixes (coffee whitener), dairy based drinks (UHT applications, coffee) sports nutrition and weight-loss beverages, protein bars, sauces, toppings, as well as processed cheese products.

The composition and protein level is a determining factor in choosing the best MPC (Milk Protein Concentrate). One of the most important analysis in food additives is the determination of Nitrogen and protein content applied for quality control and R&D purpose. So, for this the use of an accurate and automatic analytical techniques which allows the fast analysis with an excellent reproducibility is required. The FLASH 2000 Analyzer, based on the dynamic flash combustion of the sample, copes effortlessly with the wide array of laboratory requirements such as accuracy, day to day reproducibility and high sample throughput. This alternative to the classical Kjeldahl method, based on Dumas (combustion) method, has been developed and approved by different associations.

This paper presents data on Nitrogen/Protein determination in different milk protein concentrates and isolates, obtained with the analyzer to demonstrate the validity of the method without matrix effect analyzer.

### Keywords:
- Elemental Analysis
- Protein
- Quality Control

### Application Code:
- Food Contaminants

### Methodology Code:
- Other
N-protein analysis based on the Dumas principle relies on quantitatively converting the sample of interest into the gas phase at elevated temperatures in the presence of elemental oxygen. Excess oxygen is commonly bound on metals such as copper or tungsten prior to chromatographic separation of the combustion gases followed by detection of N\textsubscript{2} correlating to protein content. The reaction of copper or tungsten to the according metal oxide throughout the course of several hundred analyses causes deficiency in oxygen binding as well as insufficient conversion of NO\textsubscript{x} to N\textsubscript{2}. Thus, erroneous nitrogen / protein values results. In order to circumvent these effects a frequent exchange of so-called reducing metals is required. Here, we present a unique method to recover the metal oxide to the active metal species increasing the life time. Experimental results clearly depict an approx. fivefold increase in life time of the reducing metal while maintaining outstanding precision in nitrogen / protein values results.

Keywords: Analysis, Elemental Analysis, Food Identification, Food Science
Application Code: Food Identification
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Session Title: Improvements in Forensic Analysis

Abstract Title: Analytical and Synthetic Studies on Substituted Cathinones: Bath Salt-Type Aminoketone Designer Drugs

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Abstract Text:
This presentation will describe our research efforts to evaluate the structure-retention, structure-fragmentation and other structure-property analytical relationships for a large series of substituted aminoketones related to the cathinone-type drugs.

Our research has focused on the development of regioisomer specific methods for the identification of ring substituted aminoketone compounds (cathinone derivatives). The work includes the chemical synthesis of all regioisomeric forms of selected aromatic ring substituted aminoketones; generation of analytical profiles for each compound; chromatographic studies to separate/resolve all regioisomeric aminoketones having overlapping analytical profiles, and design and validation of confirmation level methods to identify individual compounds.

Based on the structure of the unsubstituted cathinone molecule, designer modifications are possible in three distinct regions of the molecule: the aromatic ring, the alkyl side chain and the amino group. Example compounds from all three of these areas of designer modification have been reported as components of clandestine drug samples. Commercially available precursor aldehydes, alkyl halides and amines can be converted to a wide variety of cathinone-type compounds. Legal control of a specific molecule often provides the driving force for clandestine development of additional substituted cathinone designer molecules.

The synthesis, GC-MS, IR and related spectroscopic properties will be presented for several series of substituted cathinone derivatives.

Keywords: Forensics, Gas Chromatography, GC-MS
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography/Mass Spectrometry
Abstract Text

Current methods for the extraction and concentration of ignitable liquids from fire debris utilize passive headspace concentration with activated carbon strips (ACS). Typical extractions are conducted between 60-80°C for 12-16 hours based on ASTM guidelines followed by a time consuming GC/MS analysis. A New Rapid Approach: Dynamic Headspace Sampling - Sorbent-Coated Mesh - DART-Mass Spectrometry method has been developed. Gasoline from seven different vendors was analyzed directly by direct analysis in real time (DART-MS). An evaporation series of gasoline consisting of 0%, 25%, 50%, 75% and >90% was created for each of the seven brands of gasoline. Following the analysis of the neat samples, a modified dynamic headspace sampler using a novel, sorbent-coated mesh for the selective capture of target analytes from fire debris samples was evaluated. A rapid, 5-minute, heated extraction requiring minimal sample preparation for the detection of the isolated species from ignitable liquids was accomplished. DART-MS generated spectra rich in ions characteristic of both hydrocarbons and additives were detected within the mass range of 50-1,000amu. Chemometric Analysis was used to generate statistical models built utilizing analytical data generated from the different vendor brands of gasoline. The weighted spectral linear chemometric algorithm was successful in identifying and categorizing each gasoline sample with a 0% error rate after running a 10 fold, 5 run cross validation on the samples. Statistical analysis of the generated mass spectral profiles obtained by DART-MS resulted in discrimination of the analyzed samples in a fraction of the time in comparison to traditional methods. The speed and efficiency associated with both the sample preparation and the DART-MS analysis, in combination with statistical methods and model building approach, demonstrates an attractive analytical approach for the rapid analysis of ignitable liquids from fire debris samples.

Keywords: Forensic Chemistry, Fuels\Energy\Petrochemical, Mass Spectrometry, Portable Instruments
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Mass Spectrometry
Improvements in Forensic Analysis

Analytical Studies on 1-n-pentyl-naphthoylinodes: Regioisomeric Compounds Related to the Synthetic Cannabinoids

Many of the synthetic cannabinoid drugs of abuse are 1-alkyl-3-acyl indole derivatives. One of the more common drugs in this series is JWH-018, 1-n-pentyl-3-(1-naphthoyl)indole. This report compares the GC-MS, HPLC and FT-IR properties of all 6 regioisomeric 1-naphthoyl and all 6 regioisomeric 2-naphthoyl substituted-1-n-pentylindoles. These compounds have the 1- and 2-naphthoyl-group attached at each of the 6 possible ring substituent positions of the indole ring. The twelve compounds have the same elemental composition C24H25NO yielding identical nominal and exact masses. Additionally, the substituents attached to the indole ring, 1- and 2-naphthoyl- and 1-n-pentyl-groups, are identical for all twelve isomers. The electron ionization mass spectra show equivalent regioisomeric major fragments resulting from cleavage of the groups attached to the central indole nucleus. Fragmentation of the naphthoyl and/or pentyl groups yields the cations at m/z 234, 220, 214, 186 and 144. While the relative abundance of the ions varies among the regioisomeric substances a number of these compounds share very similar relative abundances for the major fragment ions. Deuterium labeling studies were used to prepare the d11-n-pentyl-, d5-indole- and d7-naphthoyl-analogues of JWH-018 in order to confirm the EI-MS fragmentation processes for these compounds.

Chromatographic separations by capillary GC as well as HPLC provided excellent resolution of these compounds. The elution order appears related to the relative distance between the two indole substituted groups. Infrared absorption spectral data show the carbonyl absorption band for each of the naphthoylinindoles and provide distinguishing and characteristic information to help to individualize each of the regioisomers in this set of compounds.

Keywords: Forensic Chemistry, FTIR, Gas Chromatography, Gas Chromatography/Mass Spectrometry
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography/Mass Spectrometry
Improvements in Forensic Analysis

Application of LIBS-Enhancement Techniques to Uranium-Containing Materials

The rapid detection of the isotopes of uranium is important for the prevention of nuclear proliferation. Although several techniques exist for this purpose, common techniques such as mass spectroscopy require substantial sample preparation or potentially expose the equipment, the operator of the equipment, or both to radiation. Moreover, these techniques may not be able to make an assessment quickly, or they may require substantial amounts of material or high levels of enrichment to work properly.

One technique that solves these detection problems is laser-induced breakdown spectroscopy (LIBS). It can be fast, needs only sub-micrograms of material for an analysis, requires no sample preparation, and can be man-portable. In addition, it could be operated in a standoff regime to ameliorate some of the effects of radiation on the operator and equipment with the proper instrument design. These advantages make LIBS suitable for site-surveying of uranium, which can be difficult with currently available methods.

Though LIBS can detect uranium, as well as its isotopes under certain conditions, it can benefit from enhancement techniques that allow for lower levels of detection of uranium overall and better precision for isotopic abundance. More routine LIBS-enhancement techniques like double-pulse LIBS (DP-LIBS) can improve the overall emission, and techniques like LIBS with laser-induced fluorescence (LIBS-LIF) can improve the relative isotopic-abundance accuracy. Other enhancement methods, such as Townsend-effect plasma spectroscopy (TEPS), resonant LIBS (R-LIBS), and resonant-enhanced LIBS (RELIBS), can potentially improve the detection limits or relative abundance precision even more, though their application to uranium has been limited or is non-existent. Consequently, these latter techniques are chosen for study here, and the results of these techniques on potential detection-limit or relative-abundance accuracy improvements are discussed in this work.

Keywords: Atomic Spectroscopy, Isotope Ratio MS, Plasma Emission (ICP/MIP/DCP/etc.)
Application Code: Homeland Security/Forensics
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Multivariate Classification Model Transfer for the Discrimination of Textile Fibers by UV-Visible Microspectrophotometry

Abstract Text

Ultraviolet (UV)-visible microspectrophotometry has been used for decades as means for discriminating metameric fibers in forensic casework. Recent studies have shown that multivariate classification techniques are an effective tool for characterizing such fibers. The ability to transfer classification models between laboratories could save time and resources in forensic analyses. However, issues transferring models of this type from one laboratory to another can arise as a result of differences in sample preparation, environmental conditions, and instrumental signal response.

In this study, UV-visible absorbance spectra of 12 blue acrylic fibers were examined at five separate locations including three academic research laboratories and two forensic laboratories. The data received from these facilities were analytically assessed in three manners. Multivariate classification models were initially constructed on each individual laboratory’s dataset to evaluate intra-laboratory variability between samples. In a second subset of the study, discriminant analysis was performed after merging all data collected in the study. Lastly, the transferability of classification models was assessed by predicting class membership of samples analyzed at a single laboratory using models built from the spectra collected at the four remaining locations.

Principal component analysis (PCA) followed by linear discriminant analysis (LDA), quadratic discriminant analysis (QDA), or support vector machine (SVM) classification were used to evaluate the agreement of results among the laboratories. An average classification accuracy of 93.2% was found after training the discriminant analysis models using data collected at four laboratories and using the information collected at the fifth laboratory as an external test set. For comparison, intra-laboratory studies carried out produced an average classification accuracy of 96.3%. The reduction in the discriminative abilities of the transferred models was likely due to the differences in spectral noise and peak intensities experienced between laboratories. On the whole, the errors generated by QDA were lower than those resulting from LDA and SVM.

Keywords: Chemometrics, Forensics, UV-VIS Absorbance/Luminescence
Application Code: Homeland Security/Forensics
Methodology Code: Chemometrics
Introduction: ‘Spice’ is a herbal mixture mostly sprayed with various types of synthetic cannabinoids which act as agonists at cannabinoid receptors and cause neurological effects similar to cannabis. Abuse of these herbal products is a current social problem worldwide. In this study we describe direct mass spectrometry (MS) coupled with counter-flow introduction atmospheric pressure chemical ionization (CFI-APCI) for rapid screening of synthetic cannabinoids in herbal products.

Methods: Experiments were performed using a Hitachi DS-1000 ion-trap mass spectrometer equipped with an APCI source, a metal block heater and a diaphragm pump. Small pieces of herbal products (less than 1 mg) were sandwiched between glass microfiber filters and subjected to direct analysis. Authentic standard solutions of the cannabinoids were dropped on the filter for analysis. Analytes in the samples were thermally desorbed with the heater at 320°C, and introduced to a CFI-APCI source with ambient air by the pump. Ions generated by corona discharge were sent in the direction opposite to the air flow by an electric field, and introduced into the ion-trap mass spectrometer.

Results: Authentic standard JWH-081, JWH-210, APICA and XLR-11 had the major ions corresponding to their protonated molecules at [m/z] 372, 370, 365 and 330, respectively, in the full-scan mass spectra (single MS mode). Collision-induced dissociation of protonated molecules gave characteristic product ion spectra (tandem MS mode). Lower limit of detection for 91 synthetic cannabinoids tested in this study was less 100 ng on filter (S/N ratio, >5 on MS/MS traces). Analysis of herbal products containing these compounds showed characteristic ions both in single and tandem MS modes, enough for identification of the compounds.

Conclusion: The method requires neither sample pretreatment nor separation step, and would be applicable for rapid screening of synthetic cannabinoids in herbal products.
### Abstract Text

Microorganisms have been shown to retain a chemical signature indicative of the laboratory medium used for culturing. However, the repeatability of medium-specific chemical signatures has not been demonstrated from samples of microorganisms produced in the same batch or in different batches by the same sporulation protocol. The objective is to compare the variation in Raman spectra of bacterial endospores repeatedly prepared by the same procedure to the variation between Raman spectra of spores prepared using different media. Initially, variation was assessed by a comparison of five sporulation media. Secondly, seven additional medium formulations (12 media formulations total) were measured to increase the number of sporulation media that can be identified. Using Raman spectroscopy and multivariate data analysis, initial results showed that the five media used to sporulate Bacillus cereus T-strain (BcT) were correctly identified for 100% of spores grown in a controlled manner by the same scientist. The results from BcT spores produced in 12 different sporulation media showed correct identification with 98% accuracy (with all but one sporulation medium in this data set identified with 100% accuracy). Differences were discerned between freshly prepared/freeze-dried spores and frozen spores; however, the differences did not impact the identification of the sporulation medium. Since the sporulation medium has a reproducible impact on the Raman spectrum of a BcT spore, this technique may be able to provide leads in an investigation of a criminal release of microorganisms. Thus, the significance of this work is the provision of a tool for microbial forensic analysis.

### Keywords
- Data Analysis
- Forensics
- Raman
- Sample Preparation

### Application Code
- Homeland Security/Forensics

### Methodology Code
- Biospectroscopy
Improvements in Forensic Analysis

Approaching the Ideal Forensic GC-MS

The use of 5975-SMB GC-MS with Cold EI was explored with advanced forensic applications. This unique GC-MS is based on interfacing the GC and MS with supersonic molecular beams (SMB) along with electron ionization of vibrationally cold sample compounds in SMB in a fly-through ion source (hence the name Cold EI). The 5975-SMB provides much faster analysis, enables simplified sample handling and preparation for analysis, increases the range of compounds amenable for analysis and improves sample identification via the availability of enhanced molecular ions and TAMI software, which provides elemental formulae and is automatically linked with the NIST library.

GC-MS with Cold EI approaches the ideal forensic GC-MS system via improvement of all types of forensic applications including:

A) The full range of organic explosives is amenable for analysis including the labile peroxide explosives TATP and HMTD with enhanced molecular ions and femtogram range LOD.

B) Universal very fast GC-MS analysis method serves for illicit drugs (heroin, cocain) analysis in under 3 minutes total analysis cycle time, using flow programming.

C) High throughput forensic analyses with a new Open Probe fast GC-MS provide real time analysis (20s) with separation and library identification.

D) Unknown sample identification is improved via the combination of enhanced molecular ions, extended range of compounds amenable for GC-MS analysis and the TAMI software.

E) Improved arson investigations are obtained via Isomer distribution analysis of fuels and oils for their source characterization.

F) Unique material characterization is obtained via sample specific “large” compounds and their molecular ions.

Abstract Text

Keywords: Forensics, Gas Chromatography/Mass Spectrometry, GC-MS
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography/Mass Spectrometry
Proton-Transfer-Reaction Mass Spectrometry (PTR-MS) is a very sensitive real-time trace gas detection technology and thus well established in many fields of application (environmental chemistry, food and flavor research, medical sciences, etc.). However, so far PTR-MS is predominantly used as a research tool in scientific environments and only to a minute extend for automated detection. Here we present a very recently developed method enabling not only routine detection, but also substance identification with a high level of confidence. We demonstrate the functional principle by analyzing a blend of new psychoactive substances (NPS) marketed under the lurid name "synthacaine" (i.e. synthetic cocaine) via the internet. At first, we obtain a mass spectrum of the unknown blend's headspace using H\(_3\)O\(^+\) as reagent ions and tentatively assign the major mass peaks to protonated benzocaine (anesthetic; used to create the cocaine-like numbness effect) and methiopropamine (active ingredient; stimulant; structurally related to methamphetamine; compare figure below). However, as information about the exact mass is not sufficient for the unambiguous identification of a substance, we subsequently change the E/N (reduced electric field strength) in the PTR drift tube and compare the product ion branching ratios to those we obtained from pure compounds. Furthermore, we switch the reagent ions to NO\(^+\) and again compare the branching ratios with those of the pure substances at two E/N values. The whole process takes less than 30 s, facilitates nearly unambiguous compound identification and can easily be automated.

ML and KB have been supported by the European Commission’s 7th Framework Programme (GA 287382).
The developed method for arson detection involves the concentration of the volatile fraction of fire debris in activated charcoal strips, the extraction of these compounds to a solvent, the characterization of the extract by gas chromatography-mass spectrometry (GC-MS) and the demanding interpretation of the instrumental signal [1].

The interpretation of the GC-MS signal can involve the time consuming evaluation of the chromatograph by comparing the mass spectra of relevant peaks with adequate libraries. These comparisons are usually assisted by target compound analysis and extracted ion profiling.

In order to simplify this process, chemometric techniques are proposed.

These tools are based on signals of several ILs that were burned in the presence or absence of substrates. Afterwards, three machine learning tools (MLT), decision trees, random forests, and self organizing maps were trained and optimized for the identification of unknown samples. The multivariate space was also interpreted using Principal Component Analysis and the maximum Euclidean distance to cluster centroids.

The tools were validated through the analysis of unknown samples. The reliability of the output of the tools was quantified by the examination uncertainty using Bayesian metrics [2], allowing to estimate increments in the identification quality when independent tools are combined to identify ILR in fire debris.


Keywords: Chemometrics, Forensic Chemistry, Gas Chromatography/Mass Spectrometry, Identification
Application Code: Homeland Security/Forensics
Methodology Code: Chemometrics
Development of an LC-MS Method for the Simultaneous Determination of Metformin and Miglitol in Human Plasma: Application to Pharmacokinetic Studies

Objective: The purpose of the present study was to establish a simple, fast, and sensitive method for the simultaneous estimation of metformin (MET) and miglitol (MIG) in human plasma using voglibose as an internal standard (IS).

Methodology: Chromatographic separation was accomplished using a Zorbax Eclipse C18 analytical column. The mobile phase consisting of 95% ammonium acetate (0.02 mM, pH 6.8) and 5% methanol was pumped isocratically at a flow rate of 0.5 mL/min. Selected ion-monitoring mode with molecular ions [M + H]+ at m/z 130.1 for MET, m/z 208.1 for MIG, and m/z 268 for VOG (IS) was selected for analysis. The developed method has been validated by evaluating all of the parameters specified according to the US Food and Drug Administration’s bioanalytical validation method guidelines.

Results: The calibration curve showed a good correlation coefficient during the linearity study in the range of 40 to 2000 ng/mL for MET and 50 to 4000 ng/mL for MIG. The accuracy of the proposed method for analysis of MET and MIG was 98.18% and 99.13%, respectively. Furthermore, both interday and intraday analysis showed less than 7% of the relative standard deviation. In addition, recovery of MET and MIG from plasma was found to be 94.7% and 93.1%, respectively. Subsequently the method was applied to determine the pharmacokinetic parameters such as Tmax, Cmax, AUC0–t, T1/2 and Kel for both the drugs in human plasma. Conclusions: The developed LC-MS method is the first of its kind and could be used for the simultaneous determination of MET and MIG in pharmacokinetic studies.

Keywords: Bioanalytical, Drugs, Liquid Chromatography/Mass Spectroscopy, Plasma

Application Code: Bioanalytical

Methodology Code: Liquid Chromatography/Mass Spectrometry
Anionic substances analyzed by reverse phase mode generally require pretreatment of the sample by derivatization or addition of ion pair reagent to the eluent. In this study, a multimode column (RP + anion exchange or HILIC), with polyvinyl alcohol packing material modified with quaternary ammonium functional groups was used under conditions without derivatization or ion pair reagent for the LC/MS analysis of various anionic substances.

One example of this column applies to the analysis of haloacetic acids which are common undesirable disinfection byproducts (DBPs) generated during water purification. In the past, GC/MS was used as the standard method for haloacetic acid analysis; three haloacetic acids (monochloroacetic acid, dichloroacetic acid, and trichloroacetic acid) were simultaneously analyzed by LC/MS with the multimode column. The optimized eluent condition was 25 mM CH₃COONH₄ aq. (pH 9.2)/CH₃CN = 50/50. The flow rate was 0.2 mL/min, and the column temperature was 30 °C. ESI-MS with SIM and MRM mode was used for detection. The mixed standard solution (2 ng/mL of each) was analyzed. All peak shapes were sharp with baseline separation, and the calibration curves showed high linearity. Tap water was analyzed without interference of detection.

We will also introduce the applications of other substances (e.g. oxyhalides including perchloric acid, iodine ion, organic acids, allantoin, and phosphorylated saccharides). The multimode column is useful for the LC/MS analysis of various anionic substances using more simple conditions than previous methods.
Simultaneous Quantitative Analysis of Polar and Nonpolar Compounds in the Brain with Hydrophilic Interaction Liquid Chromatography-Triple-Quadrupole Mass Spectrometry

The brain contains a wide range of molecular types; the ability to quantify polar and nonpolar compounds from the same volume-limited sample is important in many studies. Here we study changes in a range of biologically relevant molecules depending on nutritional supplementation. We use hydrophilic interaction chromatography (HILIC) to separate diverse molecular classes. We hyphenate HILIC to a triple-quadrupole mass spectrometer (TQ-MS) to quantify hydrophilic (e.g. alanine) and hydrophobic (e.g. tocopherol) analytes from 1-2 mg of wet weight biological samples. The EVOQ UHPLC-TQ MS system (Bruker Daltonics) was used and a series of MRM methods created to quantitate compounds of interest in the mouse brain. Because of a high solubility of most targeted analytes in ethanol and the compatibility of HILIC with three-stage ethanol-water-ethanol extraction, we used this approach to reduce the number of sample steps. High throughput of analysis was achieved with an average run duration ~6 min. Linear working curves over a wide range (10 ppb to 10 ppm) for compounds of interest including the neurotransmitters such as GABA and serotonin were obtained. Although poorly separable in these experiments from other forms of tocopherols, the identification and quantitation of tocopherol from the brain samples was possible due to its high abundance, different molecular mass, as well as distinguishing MRM transitions. Changes in the levels of a range of these compounds in the mouse brain will be discussed as a function of nutritional supplementation.

Keywords: Biological Samples, Chromatography, HPLC, Liquid Chromatography/Mass Spectroscopy

Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Carboxymethyl cellulose (i.e. CMC or cellulose gum) is a fluid absorbent used in food packaging and food contact materials. CMC can also be used as a direct additive for foods and drugs in order to change texture and act as a binder. CMC and other carboxymethyl starches are synthesized by condensing glycolic acid with monochloroacetic acid. Diglycolic acid (DGA) is an impurity produced by this condensation which cannot be completely removed. Currently there are no analytical methods to accurately detect and quantify DGA in foods and food packaging materials. Because DGA has been shown to be a potential renal toxicant, the determination of DGA available either from food packaging or from direct addition to food is needed.

Previously published methods for DGA analysis used liquid chromatography with UV/Vis detection and employed nonvolatile buffers. LC-MS/MS requires the use of volatile buffers, but allows for a larger dynamic detection range of the analyte. Multiple columns and mobile phases were tested during method development in order to achieve retention of the small polar molecule of DGA on the column. In addition, experimental conditions were optimized to ensure that the method could accurately differentiate DGA from the co-eluting isomer, malic acid. This poster will discuss the development and validation of a LC-MS/MS method for detection of DGA in a food packaging material and in food grade CMC material intended for direct addition into foods.

Keywords: Food Safety, Liquid Chromatography/Mass Spectroscopy, Method Development, Validation
Application Code: Food Safety
Methodology Code: Liquid Chromatography/Mass Spectrometry
Online databases can be used for the purposes of structure identification. The Royal Society of Chemistry provides access to an online database containing tens of millions of compounds and this has been shown to be a very effective platform for the development of tools for structure identification. Since in many cases an unknown to an investigator is known in the chemical literature or reference database, these “known unknowns” are commonly available now on aggregated internet resources. The identification of these types of compounds in commercial, environmental, forensic, and natural product samples can be identified by searching against these large aggregated databases querying by either elemental composition or monoisotopic mass. Searching by elemental composition is the preferred approach as it is often difficult to determine a unique elemental composition for compounds with molecular weights greater than 600 Da. In these cases, searching by the monoisotopic mass is advantageous. In either case, the search results can be refined by appropriate filtering to identify the compounds. We will report on integrated filtering and search approaches on our aggregated compound database for the purpose of structure identification and review our progress in using the platform for natural product dereplication purposes.

Keywords: Data Analysis, Data Mining, Mass Spectrometry, Spectroscopy
Application Code: General Interest
Methodology Code: Liquid Chromatography/Mass Spectrometry
Two phenylephrine (PE) unknown impurities were detected by HPLC in a commercial Cold & Flu stability samples. Their identification was confirmed by LC/MS analysis following the synthesis and structure determination of the products from the predicted interaction between PE and vanillin (component of formulation). The retention time and MS of synthesized compounds match the unknown ones.

Keywords: Characterization, HPLC Detection, Identification, Isolation/Purification
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
The natural gas from unconventional gas resources such as shale formations has gained attention due to substantial shale resources in the US, advanced extraction technologies and cost effective production. Those shale formations, such as the Marcellus Shale, are stimulated by hydraulic fracturing to allow for natural gas recovery. Hydraulic fracturing involves the injection of large volumes of water along with sand and chemical additives into the formation. The process generates large volumes of produced water that are being evaluated for potential environmental and health concerns, mainly due to implications from disposal methods and potential surface spills that could affect groundwater aquifers. Our research focuses on investigating the organic compounds present in Marcellus Shale produced water. Solid phase extraction using a Sep-Pak C18 cartridge was employed to isolate organic compounds from produced water. The different organic compounds were further eluted by organic solvents of different polarity and analysis was performed using liquid chromatography/quadrupole time-of-flight mass spectrometry (LC/Q-TOF-MS) in positive electrospray ionization mode. This presentation will summarize the mass spectral results that indicate the presence of specific organic compounds in produced waters. Further characterization of these compounds by targeted MS/MS will also be discussed.
Fatty acid esters of 2-monochloro-1,3-propanediol (2-MCPD) are toxicologically relevant contaminants formed during the industrial processing of edible oils. Currently, the only validated methods for the detection of 2-MCPD esters in edible oils are so-called “indirect” methods which require hydrolysis followed by chemical derivatization prior to analysis. These methods do not provide information about the structures of the intact esters as they occur in edible oils. Identification and quantitative information for individual 2-MCPD esters is required in order to perform an accurate risk assessment hence, the development of a quantitative method which detects intact 2-MCPD esters is needed.

The approach described herein involves rapid solid-phase extraction (SPE) cleanup of solid and liquid oils followed by LC-MS/MS detection using electrospray ionization for quantitation of intact 2-MCPD esters in edible oils. This rugged, sensitive and specific method allows for the direct determination of fatty acid esters of 2-MCPD. The method is then applied to edible oils samples to determine the occurrence of 2-MCPD esters in edible oils in the United States.

Keywords: Food Contaminants, Food Science, Liquid Chromatography/Mass Spectroscopy, Process Control
Application Code: Food Contaminants
Methodology Code: Liquid Chromatography/Mass Spectrometry
Tea is the second most consumed beverage in the world behind water. Catechins are polyphenols with antioxidant properties found in high amounts in tea, especially green tea. Green tea beverages and dietary supplements containing green tea extracts are often marketed for health benefits. Current popular methods for quantification of catechins use liquid chromatography (LC) with ultraviolet or electrochemical detection. These methods typically have long analysis times of 20 to 60 min. A reverse phase gradient elution LC method was coupled to electrospray tandem mass spectrometry to quantitate catechins in green tea beverages and dietary supplements. A method was optimized with a 3.5 minute cycle time for rapid determination of eight common catechins. Methods with external standard and internal standard calibrations were evaluated for reproducibility. The figure shows an example of a typical chromatogram of catechins from a green tea beverage.
The use of Time of Flight for screening is becoming more common in many fields. With accurate mass the number of formula can be greatly reduced. In this series of experiment we will look at a performance enhancing product for regulated drugs. To add additional confirmation to our screening, we will use a technique of varying the fragmenting voltage to produce Ms/ms like data.

Abstract Text

The use of Time of Flight for screening is becoming more common in many fields. With accurate mass the number of formula can be greatly reduced. In this series of experiment we will look at a performance enhancing product for regulated drugs. To add additional confirmation to our screening, we will use a technique of varying the fragmenting voltage to produce Ms/ms like data.

Keywords: Drugs, Liquid Chromatography/Mass Spectroscopy, Natural Products, Time of Flight MS

Application Code: Consumer Products

Methodology Code: Liquid Chromatography/Mass Spectrometry
This paper describes a simple high-throughput LC/MS method for the determination of glyphosate, AMPA, and glufosinate in milk. One milliliter of milk was shaken with three milliliters of water containing EDTA and acetic acid to precipitate protein for 10 min. After the centrifugation, the supernatant was passed thru an Oasis HLB SPE to retain suspended particulates and phospholipids. The sample extract was then injected directly to a mixed-mode HPLC column. The analytes were detected by a Sciex 5500 LC/MS/MS system in a negative mode. The internal standard was used to correct for matrix suppression. The average recovery for all analytes at 0.025, 0.1, 0.5, and 2 ppm (n= 7) are between 97-106% with relative standard deviation is less than 9%.
Arachidonic acid is acted upon by lipoxygenase enzymes, which add a hydroxyl group at 3 different positions on arachidonic acid (corresponding to the 3 different lipoxygenase enzymes, 5-lipoxygenase, 12-lipoxygenase, and 15-lipoxygenase). The products of arachidonic acid that we are interested in include 15-HETE, 5-HETE, and 12-HETE. Linoleic acid is acted upon by 15-lipoxygenase, which produces 13-HODE. Our method involves a liquid-liquid extraction using ethyl acetate of 15-HETE, 5-HETE, 12-HETE, and 13-HODE from cell debris, drying under nitrogen, and reconstitution in methanol. Cell media samples are subject to solid-phase extraction (SPE) using 85/15 hexane/ethanol elution, dried under nitrogen, and reconstituted in methanol prior to LC-MS/MS analysis. The LC-MS/MS experiments are performed using a Shimadzu IT-TOF instrument and involve a direct MS/MS method in negative electrospray (-ESI) mode, monitoring m/z 295 and 319. Chromatographic separation of the analytes is achieved using a gradient elution (A: 30% acetonitrile/70% 0.1% formic acid in water/B: 0.1% formic acid in acetonitrile) on a 250 x 4.6 mm Beckman Ultrasphere ODS column running at 0.500 ml/min flow rate. The dynamic range for the method is 0.5 – 4.5 micrograms/ml. The S-stereoisomers are the primary products when AA or LA are oxidized by human lipoxygenase enzymes, and are thus the targets of this assay. This method has been successfully applied to the study of 15-HETE, 5-HETE, 12-HETE, and 13-HODE in colon cancer cell lines HCT-116 following treatments of gamma tocopherol, gamma tocotrienol, and 95/5 gamma/alpha tocopherol.
The identity and function of many neuromodulators have been found to be largely conserved across species, including both vertebrates and invertebrates. Using the model system of the crab Cancer borealis, we wish to better understand the roles neuromodulators play in regulating biological systems. In the crab, the pericardial organs have been shown to release a variety of neuromodulators into the circulatory system (hemolymph). We focus on the roles of biogenic amines, a class of modulators including dopamine and serotonin. These amines have been demonstrated to have important roles in regulating the functions of biological systems. We are investigating the effects amines have on the stomatogastric nervous system, a likely target for these amines, in a similar manner to our laboratory’s past research on the hormonal role of GABA on stomach muscle contraction (Suljak et al., Biol. Bull. 2010, 218: 293-302). We have developed a microdialysis method to sample amines in vivo in real time, as well as multiple methods to detect and quantify amine concentrations, including liquid chromatography-tandem mass spectrometry. We have detected all amines of interest from standard aqueous and saline dilutions down to concentrations of 100 nM. We have confirmed the presence of two amines from in vivo dialysate samples and are in the process of confirming others. Results suggest the neuromodulators are present in in vivo dialysate samples, and quantifying baseline levels of each neuromodulator along with relating the dynamic nature of neuromodulator concentrations in response to different stimuli are our current focus.

Keywords: Bioanalytical, Biological Samples, Liquid Chromatography/Mass Spectroscopy
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
The development of new agricultural chemicals requires the identification of a variety of trace level unknown environmental metabolites. Some of the most challenging problems in compound development are encountered in the area of metabolite identification from complex environmental matrices. These unknowns are generally present at ultra-trace (low pg) levels, precluding their identification with NMR. In most cases, their initial identification must be performed using high resolution/accurate mass LC/MS. We will describe our use of several new approaches for accomplishing this identification using UPLC/MS (AB Sciex 5600 QqTOF) as well as the use of Ultra High Resolution NanoLC-QqITOT instrumentation (Thermo Fusion) to separate and detect the unique isotopic fine structure of these isotopically-labelled metabolites.

Metabolites were generated soil, plant cell culture, and whole plant systems. These samples were analyzed using UHPLC separations on several LC/MS systems including: 1) a Sciex 5600 (QqTOF), and 2) a Thermo Fusion (QqITOT) ‘tribrid’ instrument configured for both nano-infusion and nanoLC separations. Radiolabeled metabolites were tracked using online (RAM) detection, and ESI ionization combined with accurate MS, and MS/MS data acquisition was performed on the 5600. When required, the fractionated LC effluent was collected and assayed using a TopCount radiochemical counter to localize fractions containing the peaks of interest. These fractions were then analyzed using either an Advion Nanomate or an Eksigent 2-D nanoLC operating in trap and elute mode. These were coupled to the Thermo QqITOT instrument operating MS, MS/MS, and MSn modes at resolutions up to 450,000 (FWHM). We will present several examples where we have obtained employing these methodologies on these instruments in the identification of xenobiotic metabolites.
Abstract Text
In today’s world, there are many manufacturers offering sample preparation products and HPLC columns on the market. Choosing the most suitable sample preparation technique and the right HPLC column to develop a robust method is a big analytical challenge. As systems are becoming more and more sensitive, an efficient sample clean-up is crucial to remove interference which could alter results. This is why SiliCycle introduces SiliaPrep(X) 96 well development plate to help quickly screen which sorbent needs to be used.

Once a method is developed and validated, reproducibility is extremely important. Almost all HPLC columns on the market present a non-negligible variation from one lot to another, especially with specialty phases like ion exchangers. This is really problematic for the chromatographer as the development of robust method is extremely hard. With the new generation of SiliaChrom® HPLC columns, fluctuation is minimized and when a method is developed, you will be able to use it over and over. The tightly controlled proprietary grafting processes, combined to the unique and consistent packing processes, achieve uniform and stable bed for long lifetime and reproducibility. Recently, we introduced to new bonded phases to this series: SCX and pentafluorophenyl (PFP).

In this poster, the combination of the use of SiliaPrep(X) development plate with the new SiliaChrom phases will be presented along with a complete study on performance and stability.

Keywords: HPLC, HPLC Columns, Liquid Chromatography/Mass Spectroscopy, Sample Preparation
Application Code: General Interest
Methodology Code: Liquid Chromatography/Mass Spectrometry
Superficially porous particles (commonly referred to as SPP or “core-shell” particles) have been proven to provide fast and efficient separations. These particles feature a solid, impermeable core enveloped by a thin, porous layer of silica that decreases the diffusion path and reduces peak dispersion. As a result, significant improvements in efficiency and sensitivity can be achieved over fully porous particles of similar dimension.

In this presentation the performance of 5 µm SPP particle columns will be compared to columns packed with traditional 5 µm and 3 µm fully porous particles (FPP). The relationship between pressure and efficiency will be explored. In addition, run time, signal to noise ratio, peak width, and resolution will be evaluated in several chromatographic experiments. Each experiment will be performed on the same instrument using identical method conditions for each particle.

Through these experiments we hope to demonstrate the following advantages of 5 µm SPP particle columns over columns packed with traditional 5 µm and 3 µm FPP particles. When used in the development of new assays, they allow the chromatographer to obtain fast run times and excellent method performance without changes in instrumentation. When substituted into existing methodologies which utilize 5 µm and 3 µm FPP columns, 5 µm SPP columns have the potential to dramatically decrease analysis times while improving efficiency and sensitivity.

Keywords: HPLC Columns, Liquid Chromatography/Mass Spectroscopy, Method Development, Particle Size and Application Code: Other Methodology Code: Liquid Chromatography/Mass Spectrometry
Optimization of a Platform Method for Characterization of Intact Proteins by RP-LC-MS Using a Standard Protein Mix

Analytical labs are increasingly tasked with performing characterization of recombinant proteins. In principle, RP-LC-MS of intact proteins can rapidly provide information about the purity, sequence fidelity, post-translation modifications, and conformation. In practice, generalized methodologies and system suitability metrics are lacking for such applications. In this study, we aimed to develop a platform workflow for intact protein analyses by RP-LC-MS using a standard protein mix and define quality metrics for routine use.

For this study, more than 25 intact proteins were screened to qualitatively assess their reversed phase and MS behaviors. Selection for the final protein cocktail was based on several criteria including: relative retention time to cover a range of hydrophobicity; molecular weight spanning an approximate range of 5,000 Da to 80,000 Da; the presence of post-translational modifications, such as sialyated glycans; ease of electrospray ionization; and the quality of deconvoluted mass spectra produced. All separations were performed using BIOshell A400 Protein C4 columns packed with 3.4 micron fused core particles with 400 angstrom pore size. These fused core particles are known to enable high efficiency separations of proteins by reducing analyte diffusion in and out of the particle while maintaining typical HPLC back pressures and short run times.

Methods were developed on 2.1mm and 200um ID columns using traditional and capillary flow rates, respectively, to accommodate a variety of available instruments and sample amounts. LC conditions including mobile phase, flow rate, and gradient profile were varied. ESI source conditions, such as temperatures and voltages, and deconvolution parameters were also optimized. The generalized RP-LC-MS platform can be applied to analyte proteins in an unbiased manner and the standard protein mix enables system suitability and processing parameters to be established for proteins of various sizes.

Keywords: Biopharmaceutical, Liquid Chromatography/Mass Spectroscopy, Protein
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Liquid Chromatography/Mass Spectrometry
Vitellogenin (VTG) is an egg yolk precursor protein expressed in the females of nearly all egg laying species including fish. The expression of VTG in fish can be used as a molecular marker of exposure to estrogenic endocrine disrupting chemicals (EDCs). Enzyme-linked immunosorbent assay (ELISA) is the conventional method for measuring VTG in fish, which requires expensive ELISA kits to be purchased, and more importantly, the commercial ELISA kits are not available for all fish species. This work aims to develop a generic method to accurately measure the concentration of VTG, in more than one fish species using liquid chromatography coupled with mass spectrometry (LC/MS), which offers an accurate, cost effective and efficient way of quantifying VTG through measuring the peptides digested from VTG. A signature peptide was selected through running digested VTG standards for two fishes—fathead minnow and carp—on a 12 Tesla high resolution Fourier transform ion cyclotron resonance mass spectrometer. Fish serum samples were run through SDS-PAGE to obtain separated VTG from other proteins, then the VTG band went through in-gel digestion, and finally the VTG was quantified by measuring the signature peptide on LC/MS. The method was applied to fish serum samples from fish being exposed to different levels of EDCs. Fish samples exposed to EDCs was also analyzed by ELISA, and the results from two analyses were compared and discussed.

Keywords: Bioanalytical, Environmental, Liquid Chromatography/Mass Spectroscopy, Protein

Application Code: Environmental

Methodology Code: Liquid Chromatography/Mass Spectrometry
Injectable Hydrogels for Controlled Release of Drugs

Hydrogels have gained considerable attention as a controlled-release system for drugs. We have synthesized a transparent hydrogel with three-dimensional fibrous networks by the gelation of sodium deoxycholate in aqueous solution. Doxorubicin (DOX, a cancer drug) is loaded into the hydrogel with the swelling process. We find that the DOX loaded hydrogel can be injected into aqueous solution as hydrogel fibers through a syringe. The release rate of DOX from the injected hydrogel fibers can be tuned by altering the pH of aqueous solution.

Keywords: Biomedical, Characterization, Drug Discovery, UV-VIS Absorbance/Luminescence
Application Code: Materials Science
Methodology Code: UV/VIS
Particle size analysis of dry powders by laser diffraction offers many advantages. The technique is fast, reliable, and can be used for analyzing a wide range of particle sizes. One question that arises in these measurements is the value of the refractive index, RI, for a sample. As such, the implications of errors in this choice are an important part of understanding the uncertainty in a result. Understanding sources of and magnitude of uncertainty is important to improving measurement accuracy. For many particle systems the determined size distribution is a function of the RI used in calculating the size distribution. That is, if an incorrect value of RI is chosen, the obtained particle size distribution will be incorrect and that error is a function of the RI choice. In the historically more common analysis of samples in liquid dispersants, the RI effect is smaller than the case of dry powder analysis since the RI of air is effectively 1 and therefore smaller than values of common liquids (1.3 to 1.5). Here, the effect of RI choice on determined particle size is quantitatively analyzed for samples measured both as a dry powder and in a liquid suspension. Interestingly, even when the effect of RI is negligible, the Mie model gives better results than the Fraunhofer model. This data can be used to obtain a rough idea of the sensitivity of determined size distribution to the value of the RI used in the analysis.

**Keywords:** Particle Size and Distribution, Light Scattering

**Application Code:** Materials Science

**Methodology Code:** Physical Measurements
An ability to alter the properties of the microfluidic channel walls via introduction of nanostructures is of high-importance for lab-on-a-chip applications. Nanotexturing has been used to control material hydrophobicity, increase biomolecule-surface interactions, and control cell attachment and growth. Many of the nanotexturing techniques are expensive, labor intensive, and can only produce nanostructures at the bottom of the microfluidic channel. To address these limitations, our group has recently developed a novel technique for the fabrication of truly 3D nanostructured (3D-NS) surfaces poised within microstructures. 3D-NS micromolding masters were fabricated in impact modified (IM) PMMA using hot-embossing followed by O$_2$ plasma etching. Poly(dimethylsiloxane) (PDMS) transfer molds were then prepared via casting using 3D-NS masters as templates and were subsequently used for single step hot-embossing of both micro and nanostructures into variety of thermoplastic materials. NS surfaces were characterized using SEM, AFM, and contact angle measurements. It has been observed that the exposure of two phase material, such as IM-PMMA, to O$_2$ plasma produces NS surfaces with the average roughness in the range of 10 – 80 nm depending on the exposure time. Nanostructuring was equally effective on flat surfaces and on preformed microstructures due to isotropic character of the unbiased plasma. Flexible character of transfer molds prevented shearing off of nanostructures during de-molding step. 3D-NS microchannels were evaluated for variety of applications including isolation of circulating tumor cells (CTCs), isolation of nucleic acids, and cell cultivation.

**Keywords:** Bioanalytical, Lab-on-a-Chip/Microfluidics, Materials Science, Nanotechnology

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
High-temperature liquid chromatography (HTLC) is recognized today as a valuable technique in reversed phase high-performance liquid chromatography (RP-HPLC). Some aspects probably limit the use of high temperatures, including the lack of commercially available instruments equipped with ovens (1). Since high temperature liquid chromatography requires elevated temperature, an associated concern is whether the stationary phase is thermally stable (2). In order to develop more stable packing material for high-temperature liquid chromatography (HTLC), a new stationary phase, 1-naphthylamine attached poly (2-hydroxyethylmethacrylate-N-methacryloyl-(L)-histidine-methyl-ester) (NA-PHEMAH), was investigated in this work. The temperature used was 150 oC depending on the working conditions. The column was evaluated for 6000 – 18000 column volumes at each elevated temperature. Retention factors and plate numbers were calculated for each chromatogram at a given column volume to monitor any degradation of the stationary phase under high temperature conditions. The results demonstrate that the new NA-PHEMAH stationary phase is more suitable for high-temperature liquid chromatography separations with a good thermal stability.

References

Acknowledgements
This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK): grant number KBAG-112T336.
There has always been an interest in the rosin extracted from pine and other trees, due to its wide spread use from musical instruments to printing inks to fluxes. In last many years people have started to modify the rosin to enhance certain properties. This has resulted in many varieties of modified rosin also available for applications. In order to keep up with the changes, analytical techniques are also getting modified. Initially only FTIR was the method for knowing the type of rosin. Now FTIR is used as a qualitative tool, but other modern techniques like GC, MALDi are used for detailed exploration of rosins.

We present a method to compare various types of rosin using Gas Chromatography, and quantify them in mixtures. We have compared six types of rosin including natural rosin and modified rosins. Rosin being of natural origin are mixtures, and it is a challenge to analyse and quantify. The lowest limit of detection for rosin is 100ppm.

The instrument used is Agilent GC equipped with FID detector and DB-1 column 30mt x 0.32mmid. Using standard setting the method has been validated as per ICH guidelines. The correlation coefficient is calculated to be 0.998 with accuracy and precision between the acceptable limit of 90-110%. One step derivatization is used before analysis by GC. Using the above method many in-house samples as well as commercial samples have been analysed, and individual rosin acids have been quantified. The results have been very useful in research and development and have been applied for routine testing of rosin.

**Keywords:** Analysis, Calibration, Derivatization, Gas Chromatography

**Application Code:** Materials Science

**Methodology Code:** Gas Chromatography
USPD is a cost effective ultrasonic backscatter means to measure concentration and particle size distribution with resolution comparable to TEM that can be used in a batch mode or, by virtue of its single-transducer operation, inserted into existing equipment. With USPD, particles in suspension insonified by narrowband unidirectional ultrasound are accelerated into motion away from the transducer. Velocities measured by Doppler shifts in the backscatter collected by the same transducer for particles in the range 5 – 100 nm vary inversely with particle size. To calibrate the system, size distributions of identically prepared samples of polystyrene and colloidal silica (Ludox) particles determined by TEM have been correlated with backscatter USPD spectra. To compare these measurements, TEM-determined PSD’s have been converted to "expected" backscattered USPD spectra by multiplication of the number in each TEM histogram bin by particle diameter to the sixth power based on the Rayleigh-scattering dependence of scattering on particle size. Calibration curves for polystyrene and silica (Ludox) particles so constructed are very similar, suggesting that calibrations for a variety of materials may be comparable. A historical limitation of USPD has been a requirement for relative high concentrations. Recent work to modify and upgrade system hardware and software, presented here, has reduced the system noise floor by a factor of about 300, allowing for the measurement of PSD on samples with concentrations less than one percent.

Keywords: Nanotechnology, Particle Size and Distribution, Process Control, Process Monitoring
Application Code: Nanotechnology
Methodology Code: Physical Measurements
The complexes of the type $[ML_2]$ and $[ML'LCl]$ where $M$=Cr(III), Mn(II), Co(II), Ni(II), Cu(II) and VO(II), $L$=tridentate ligand (ONS) o-carboxyl-N-phenyl-amido thiophene and $L'$= heterocyclic N-base such as 2,2'-dipyridyl or 1,10-phenanthroline. All the complexes isolated in solid state, are stable in air and characterized by elemental analysis, metal content determination, FT-IR, $^1$H-NMR, electronic spectra, magnetic measurements, thermogravimetric analysis (TGA/DTG). The physicochemical data suggest a square pyramidal structure of VO (II), pseudo octahedral to Cu(II) and an octahedral for Cr(III), Mn(II), Co(II), and Ni(II) complexes. The ligand field parameter have been calculated and related to the electronic environment. The tridentate ligand $(L)$, binary and ternary complexes were screened for their antimicrobial activities against various bacteria.

**Keywords:** Materials Characterization, Spectroscopy, Thermal Analysis

**Application Code:** Materials Science

**Methodology Code:** UV/VIS
Material Sciences

Comparative Study of Saturation Effect in Laser Induced Breakdown Spectroscopy (LIBS) and Laser Induced Molecular Emission Spectroscopy (LIMES)

Laser induced breakdown spectroscopy (LIBS) is widely used laser spectroscopic techniques in various fields, such as material science, forensic science, biological science, and the chemical and pharmaceutical industries. In most of LIBS work, the analysis is performed using radiative transitions from the atomic emissions. Recently, some work on Laser Induced Molecular Emission Spectroscopy (LIMES) has started. The LIMES analysis was performed using radiative transitions from the molecular band emissions. In this work, the comparative study of saturation effect in LIBS and LIMES is performed to obtain the optimum experimental condition to lessen the saturation problems. A binary mixture of strontium oxide and aluminum oxide of different concentrations in powder form was used as sample. LIBS and LIMES spectra were collected by varying various parameters, such as laser energy, gate delay, and gate width to optimize the LIBS and LIMES signals. The atomic emission from Sr and Al lines observed in LIBS spectra of different sample composition were used to characterize the laser induced plasma and correlate the effect of saturation.

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Abstract Text
Laser induced breakdown spectroscopy (LIBS) is widely used laser spectroscopic techniques in various fields, such as material science, forensic science, biological science, and the chemical and pharmaceutical industries. In most of LIBS work, the analysis is performed using radiative transitions from the atomic emissions. Recently, some work on Laser Induced Molecular Emission Spectroscopy (LIMES) has started. The LIMES analysis was performed using radiative transitions from the molecular band emissions. In this work, the comparative study of saturation effect in LIBS and LIMES is performed to obtain the optimum experimental condition to lessen the saturation problems. A binary mixture of strontium oxide and aluminum oxide of different concentrations in powder form was used as sample. LIBS and LIMES spectra were collected by varying various parameters, such as laser energy, gate delay, and gate width to optimize the LIBS and LIMES signals. The atomic emission from Sr and Al lines observed in LIBS spectra of different sample composition were used to characterize the laser induced plasma and correlate the effect of saturation.

Keywords: Atomic Spectroscopy, Quantitative, Spectroscopy
Application Code: Materials Science
Methodology Code: Molecular Spectroscopy
Synthesis and Characterization of Stable, Long-Lived Carbazole-Derived GUMBOS (Group of Uniform Materials Based on Organic Salts) for Organic Light Emitting Diodes (OLEDs)

Organic light-emitting diodes (OLEDs) are semiconductor devices that use thin films of organic molecules as the emissive electroluminescent layer. Displays with OLEDs do not need backlighting such as liquid crystal displays (LCDs), hence save tremendous amount of energy. In this research, a simple synthetic protocol was applied to synthesize carbazole-derived GUMBOS (Group of Uniform Materials Based on Organic Salts) as potential candidates for emissive material in OLEDs. One group of synthesized compounds has a substituted carbazole as the cation and a bis(pentafluoroethylsulfonyl)imide (BETI) as the anion, which emits a single color of light. The other type also has a substituted carbazole as the cation and an anion which is also capable of electroluminescence such as Fluorescein. The latter group of compounds emits more than one color of light. Evaluation of their photostability and thermal stability showed these compounds are sufficiently resistant to photo-bleaching and thermal degradation, which are the most common problem associated with OLEDs. Calculations of the valence and conductance band gap (Eg), HOMO and LUMO energy levels, and quantum yields also indicated that they can be successfully applied as emissive layers of OLEDs.

This research is supported by the National Science Foundation under grant no.CHE-1243916.
Dielectric Measurements of Materials Embedded in Polymer Matrixes with Terahertz Time Domain Spectroscopy

Terahertz time domain spectroscopy (THz-TDS) has the capacity to explore intermolecular interactions and lattice vibrations of solids, thereby providing a means to understand the structural properties of materials. In addition, THz-TDS has the potential to probe dielectric properties of materials at frequencies much higher than conventional MHz methods. Dielectric measurements are difficult at any frequency when sample dimensions are small, as is common for crystals and nano-particles.

We are interested in establishing the utility of dielectric measurements for small dimensional materials at THz frequencies. These measurements involve transmitting coherent THz radiation through a sample and determining the dielectric properties from changes in the phase angle of the transmitted radiation relative to air. Our primary interest is the accuracy of such dielectric measurements for binary samples where the material of interest is embedded within a polymer matrix with known dielectric properties. Landau, Lifshitz, and Looyenga have proposed a model (LLL-model) for such binary mixtures where the dielectric constant of the sample is the volume-weighted sum of the dielectric properties of the individual components.

This presentation will focus on accuracy of dielectric constant measurements for solid materials embedded in pellets prepared by compressing a mixture of the solid material in a polymer matrix of either high density polyethylene or Teflon. Materials tested include Mg(OH)2, Al2O3, and ZnO, for which the dielectric constants are known at THz frequencies. Results for our THz TDS measurements coupled with the LLL-model demonstrate accurate dielectric constants for these materials.

Funding for this research was provided by the University of Iowa.

Characterization of colloidal systems and investigation of their stability in their native state (i.e. without denaturation) is of prime importance for the formulator who wants to optimize the development of new products. A new technique has been developed, based on Multiple Light Scattering (MLS), to fulfill this purpose. It has been designed to measure and elucidate instability phenomena in liquid colloidal dispersions from 0 to 95% in volume fraction, with particles from 0.1µm to 1mm, 5 to 50 times quicker than the naked eye. It has also proven to be a useful technique to characterize the dispersion state of colloidal samples (for quality control purposes) and the mean diameter of the particles in the dispersion.

The Turbiscan technology is fitted with a fully automated ageing station enabling to monitor the stability of various formulations at three different temperatures and to get automatic data processing, helping the formulator to save time and test more formulations. This ageing station can be used for high throughput screening tests, determination of expiry dates or quality control.

**Keywords:** Laser, Light Scattering  
**Application Code:** Materials Science  
**Methodology Code:** Other
A simple approach for the preparation of monodisperse porous polymer particles was proposed based on the inkjet technology. A piezoelectric drop-on-demand (DOD) inkjet injector with its nozzle submerged into an organic phase was used to generate monodisperse particles. With fine tuning of the waveform applied to the piezoelectric transducer on inkjet microchip, aqueous polymer solution (sodium poly-styrenesulfonate, NaPSS) was easily ejected from the injector into the organic phase (1-butanol). Subsequently, a polymer droplet was generated in the organic phase. Due to the extraction of water from the droplet to the organic phase, accompanied with self-assembling property of polymer molecule, the volume of the droplet shrank. Consequently, the micro-scale particles yielded with a smooth polymer shell and internal porous structure. With the presented system, the porous polymer particles with a narrow size distribution can be obtained by single step. The influencing factors for polymer droplet generation in organic solvent, including polymer property, organic solvent type, and inkjet waveform, were investigated. And the relationship between final particle diameter and initial emulsion droplet size were also studied. By changing the waveform on inkjet microchip, diameters of particles could be precisely controlled from 15 μm to 60 μm with RSD of 2.7%–4.8%. Additionally, various dimensional hollow polymer particles were produced by changing the polymer solution concentration. It predicted that the smaller particle could be obtained when a smaller size nozzle of inkjet was adopted. And the high-throughput production could easily achieved by fabricating multi-nozzle inkjet setup via adding the number of nozzles and cables. We believe that the inkjet-based approach described here would become a universal platform for production of monodisperse porous polymer particles.
Silk fibroin (SF) is a typical natural biopolymer derived from cocoons of domestic silkworm, Bombyx mori. Silk protein is environmentally friendly, renewable, and non-toxic, and has unique material properties, such as tunable biodegradability and good thermal stability. In addition, it is a semicrystalline polymer with two phases, beta-sheet crystalline phase and non-crystalline structures including random coil, alpha-helix and beta-turn conformations, etc. However, regenerated SF materials with high beta-sheet crystallinity are often rigid and brittle and could not be used for soft tissues regeneration. Therefore, blending SF with other soft biomaterials needs to be developed, which can significantly improve the physical or chemical properties of regenerated SF materials. Poly(lactic acid) (PLA) is a synthetic biopolymer with good mechanical properties, biocompatibility and biodegradability etc. In this study, we blended SF with PLA systematically to produce SF/PLA composite films at different mixing ratio in chemical solvents. Differential scanning calorimetry (DSC) and Fourier transform infrared analysis (FTIR) techniques were used to investigate the molecular interactions between SF and PLA. And thermogravimetric (TG) analysis was used to evaluate the thermal decomposition temperature and the degradation rate of SF/PLA composites. This study provided comprehensive information about miscibility and stability properties of SF/PLA blend films. The SF/PLA composites can be further fabricated into various forms such as nanosphere, sponges, gels and fibers. Therefore, this study provided an important platform for the fabrication of different SF composites with various biomedical, green chemistry and engineering applications in the future.

Keywords: Biomedical, FTIR, Polymers & Plastics, Thermal Analysis
Application Code: Materials Science
Methodology Code: Thermal Analysis
In the past few years, researchers have introduced photoacid generators (PAG) in ion-selective optodes. PAG are compounds that upon exposure to an electromagnetic radiation generate protons and are irreversible, thus making them not reusable. We utilize a similar concept by incorporating photoacids (PAH), which are compounds that upon irradiation generate protons and are able to thermally reassociate their protons. This reversibility function of PAH shows promise for use in ion-selective optodes. In previous work, we showed a new type of ion sensor containing a meta-stable merocyanine PAH polymer which provides the protons for ion-exchange but also replaces traditional pH sensitive dyes (chromoionophores) and anionic sites (ion-exchangers). Herein, we have synthesized two new PAH each with a different functional group, one electron withdrawing and one electron donating. We have studied their characteristics in terms of $[\text{p} \text{i} \text{j} \text{p}]/[\text{i} \text{k}][\text{sub}]/[\text{a}]/[\text{sub}]$ change before and after irradiation in organic solution, and response time in both organic solution and ion-sensing membranes. In our findings, the functional groups attached to the PAH can have a dramatic impact on the behavior of the proton reuptake (response time) after activation. Further work is in progress to optimize the response time of PAH by understanding the fundamentals involved in these processes. We expect to design a ‘one of a kind’ ion sensor that is controlled and reusable for ion detection.

The authors acknowledge the office of Research and Commercialization, College of Sciences and the Department of Chemistry at the University of Central Florida for financial support of this research.
Recently scientific and industrial impact of nanoscience and nanotechnology in analytical electrochemistry has been growing. Electrochemical biosensors have been applied in many areas, such as food industry, agriculture, military, veterinary, clinical applications, and environment. Some challenging bioanalytical problems, such as sensitivity, specificity, reproducibility and reliability can be resolved by applying nanostructure-based electrochemical biosensors [1-4].

The glucose biosensors based on glucose oxidase (GOx) immobilized on a graphite electrode precoated by electrochemically deposited gold nanoparticles or electrochemically synthetized 3D gold nanostructures were developed. The optimal concentrations of gold nanoparticles, tetrachloroauric acid, GOx, and a redox mediator were selected, and analytical characteristics of glucose biosensors were assessed. The newly developed electrodes were successfully used for the determination of glucose in human serum samples.

Acknowledgment
This research was funded by the European Social Fund under the Global Grant Measure.


Keywords: Electrochemistry, Immobilization, Nanotechnology, Protein
Application Code: Bioanalytical
Methodology Code: Sensors
### Abstract Text

A nanoparticle platform for scintillation proximity assay (SPA) of 3H labeled analytes in aqueous environments was developed. 3H exhibits minimal effects on labeled analytes in terms of structure and function, and emits low-energy beta particles that may be detected via scintillation, a process that converts the energy absorbed from beta particles into photons emitted at visible wavelengths. The emission wavelength can be modulated by introducing one or more different scintillating fluors, or by altering the luminescent core, e.g. quantum dots, within the absorbing matrix. Such control allows wavelengths to be tuned according to application and providing means for multichannel detection. Polystyrene was used to form absorbing core nanoparticles into which one or more scintillators can be closely entrapped for effective energy transfer from matrix to scintillator. The silica shell provides amenable surface functionality for suspension stability and binding capabilities. Due to their small volume, SPA nanoparticles can potentially be used for real-time measurement of radiolabeled analytes in live cellular environments, where traditional scintillation counting techniques are impossible. SPA nanoparticles were surface functionalized with biotin to study specific detection of radiolabeled analytes using a biotin-protein binding model. Specific binding of radiolabeled NeutrAvidin to the biotinylated nanoparticles showed increased scintillation versus free 3H, resulting in specific detection of low concentration radiolabeled analytes.

### Keywords
- Bioanalytical
- Biosensors
- Nanotechnology
- Radiochemical Methods

### Application Code
- Bioanalytical

### Methodology Code
- Sensors
Controlling Valency in Antibody-Oligonucleotide Conjugate Synthesis for Proximity Immunoassays

Proximity immunoassays (PIA), including proximity ligation assays (PLA), electrochemical proximity assay (ECPA), proximity Förster resonance energy transfer (pFRET), and proximity rolling circle amplification, are recently developed protein detection techniques with high selectivity and sensitivity. PIAs take advantage of the “proximity effect” of two antibody-oligonucleotide conjugates (Ab-N) or aptamer probes that recognize a target molecule simultaneously. The oligonucleotide tails of the bound probes then trigger downstream reactions for signal amplification or direct readout. Standard methods for Ab-N synthesis yield heterogeneous products of mixtures with a range of stoichiometries, giving inconsistency and unnecessary background in Ab-N based PIAs. Here we designed a method for controlling valency in Ab-N synthesis with magnetic beads induced surface isolation and click chemistry. The approach should allow a controlled number of well-separated azide modified DNA molecules to be captured by DNA-modified beads, followed by 1:1 antibody labeling. We first introduced alkyne modifications onto antibodies by the EDC-NHS reaction, followed by click reaction with the azide-DNA. These Ab-Ns were proven functional in pFRET assays for insulin in the nanomolar range (Figure A). Furthermore, DNA-modified magnetic beads (2 µm diameter) were synthesized using click chemistry and confirmed using flow cytometry (Figure B). With these tools in place, it should be relatively straightforward to synthesize Ab-Ns in batches of controlled valency (1:1, 1:2, 1:3, etc.). In particular, monovalent Ab-Ns should exhibit significantly lower background than the currently used multivalent Ab-Ns, with only modest signal reductions.

Keywords: Bioanalytical, Immunoassay, Nucleic Acids, Protein
Application Code: Bioanalytical
Methodology Code: Sensors
The same liquid crystals (LCs) used in TV and computer screens can also be used for gas sensing. LC films provide a much simpler means to readout chemical interactions than instrumentation. A gas sensor is made by spreading a thin film of LC over a chemically functionalized surface. The detection chemistry aligns the LC molecules in one orientation before exposure to the target, and after interaction with the target it aligns the LC molecules in a different orientation. The change in LC orientation appears as a change in brightness when viewed through crossed polarizers. No power supply is needed.

This technology has been used to make dosimeters that measure ppb levels of H\(_2\)S over a period of hours. We now report an increase in sensitivity of two orders of magnitude, enabling detection of 100 ppb in less than 60 seconds of three target gases - H\(_2\)S, NO\(_2\), and NH\(_3\). These advances were made by optimizing the surface density of the chemistry that interacts with the target gas. This paves the way for highly sensitive, small, lightweight, low power gas sensors for environmental monitoring and safety applications.

This work was supported by US Army contract W911SR-11-C-0025, ARO contract W911NF-13-P-0030, and NIOSH Research Grant 1R21OH010116-01A1.

Keywords: Environmental/Air, Industrial Hygiene, Monitoring, Sensors
Application Code: Environmental
Methodology Code: Sensors
Reliable Measurements and Influence of Humidity in an Ion Mobility Spectrometer

Ion mobility spectrometers (IMS) are widely used in different applications. The ambient pressure operation of these instruments yields the dependency on several environmental parameters. At Pittcon in 2013 we have already shown the great influence of humidity on the performance of IMS and discussed the necessary consequences. In this paper we will update and confirm the results with a further method for measuring the humidity in IMS.

One major advantage of IMS systems is the operation at ambient pressure, so no vacuum system is needed. This fact enables the instrument for miniaturization and handheld operation in the field under rough non laboratory conditions. As a measurement output a time of flight or mobility spectrum is generated. The mobility $K$ of certain ions is a compound specific property and is calculated out of the drift time by $K=\frac{l}{t_d \times E}$. Up to now there is no established compensation method for humidity.

In the previous work we used, in order to measure the humidity level in the closed loop of an IMS, a moisture sensor from Michell Instruments, EA2-TX-100. The sensor based on a capacity measurement of hydroscopic layer on a ceramic substrate. In the recent work we used a sensor which based on the adsorption of water in a Phosphorpentoxid (P2O5) layer and the subsequent decomposition of the water. The sensor is operated with a defined voltage, which changes significantly with the decomposition of the water. This dependency has a close relation the humidity level and is used as an indicator.

In this paper we will compare the performance of the two humidity sensor at the boundary operating range in the lower ppm humidity level. We will show the comparison of theoretically predicted humidity levels deduce from clustering theory with the actual measurements of both sensors. The applicability of both sensors for use in an IMS as permanent sensors for humidity compensation issues will be discussed.

**Abstract Text**

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**Keywords:** Air, Sensors, Water

**Application Code:** Other

**Methodology Code:** Sensors
Volatil compounds released by microorganisms can be used to discriminate between pathogenic and non-pathogenic classes of bacterial species. Such types of discrimination could be useful in cases where one needs to rapidly detect pathogenic microorganisms in food samples in order to avoid foodborne illnesses. Amines are a product of bacterial colonization and also may be markers to other biological dysfunctions; they can be useful analytes to discriminate between different classes of foodborne microorganisms. Colorimetric reagents have been used to discriminate different analytes. They enable fast responses are cheap, readily available and easy to operate compared with sophisticated analytical methods. Cellulose acetate (CA) is an ideal matrix for entrapment of the colour indicator since this polymer presents some important characteristics like stability, homogeneity, versatility and commercial availability; in addition, it can be an efficient support for locking-in dyes and may be used in food packaging applications. Our objective has been to solvent cast CA membranes to immobilise dyes and to use the resultant indicator membranes to discriminate different types of amines. We prepared the CA membranes with five pH indicators. We then obtained images using a mounted smartphone and extracted RGB values using in-house software [1] before and after contact with analytes. All the RGB values extracted from pictures were used as input for non-supervised pattern recognition methods. With subsequent mathematical treatment, it was possible to see a clear discrimination between the amines studied (isobutyl, isopentyl and triethylamine), without any misclassification. This demonstrates that the device could be an economical and fast procedure for determination of food microbial degradation within packaged foods.

Financial support: FAPESP, CNPq and CAPES, Royal Society, UK.


Abstract Text

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Financial support: FAPESP, CNPq and CAPES, Royal Society, UK.


Keywords: Food Identification, Sensors
Application Code: Food Contaminants
Methodology Code: Sensors
In this work, fluorescent quantum dots (QDs) and gold nanoclusters (Au NCs) are confined in a drop to develop a novel sensing platform for metal ions and organometals at trace level (detection limits in the range of micro-to-nano molar). A portable micro-fluorospectrometer was employed for fluorescence measurement. CdSe and core-shell CdSe/ZnS QDs were stabilized with hexadecylamine (HDA) and dispersed in an organic drop (n-octane, decane). Metal species were incorporated into the sensing drop following headspace single-drop microextraction. This integrated system allows the isolation of the target analyte, its preconcentration and recognition in a smart way. Advantages of the novel approach include increased selectivity (phase separation removes potential matrix effects) and sensitivity as well as low consumption of nanoprobes. Consequently, the generation of toxic wastes due to elements contained in the QD composition (e.g. Cd, Se) is kept to a minimum. In addition, no functionalization of the nanoprobes is required, their dispersion in the organic solvent together with a stabilizing agent being enough for sensing. Several metal species such as selenite, methylmercury, methylcyclopentadienyl-manganese tricarbonyl, etc. quenched the QD fluorescence following the Stern-Volmer equation. Significant quenching was also observed for non-metal compounds such as hydrogen sulphide. Au NCs stabilized in an aqueous drop with sodium dodecyl sulfate (SDS) and following the same approach as above have proved to be sensitive for the detection of inorganic mercury. The novel sensing platforms may open the door to on-site trace metal analysis and speciation, with a performance similar or even better in comparison with analytical techniques typically used in central labs.

Acknowledgements: Financial support from the Spanish Ministry of Economy and Competitiveness and the European Commission (FEDER) (Project CTQ2012-32788) is gratefully acknowledged.

Keywords: Fluorescence, Nanotechnology, Sensors, Trace Analysis
Application Code: Nanotechnology
Methodology Code: Fluorescence/Luminescence
Terbium Luminescence: An Inexpensive Sensitive Probe for Detecting SNPs in DNA

Single nucleotide polymorphisms (SNPs) are the main cause of variations in human genome. SNPs arise from damaged DNA or dysfunctional replication enzymes, and have the potential to cause apoptosis, carcinogenesis, and mutagenesis. These effects have motivated the development of techniques for SNPs detection. However almost all of the proposed methods consist of multi-step procedures, are limited to specific types of SNPs, some of these methods require expensive instruments, and some suffer from high level of interferences. In this work, we present a novel simple, inexpensive, mix-and-read assay that is general for the sensitive detection of all types of SNPs. Our technique utilizes the enhancement of terbium luminescence in ssDNA regions via effective energy transfer from excited, non-hydrogen bonded bases to terbium ions (Figure One). Thus, SNPs present in the sequence of a dsDNA lead to luminescence enhancement in the presence of terbium ions. The change in luminescence is directly correlated to the number of SNPs enabling the terbium-DNA complexes to be used for sensitive quantification of SNPs in dsDNA. We optimized the experimental conditions and the position of SNPs in dsDNA for maximum sensitivity. The results show that central SNPs cause the maximum instability to dsDNA with an exponential increase in the terbium luminescence with increasing the number of SNPs in the dsDNA (Figure One). The technique’s quantification demonstrated a dynamic range of 0-72 SNPs in a dsDNA composed of 100 base pairs, with a limit of detection and limit of quantification of 3 and 9 SNPs, respectively. This assay proves that the terbium luminescence is a simple, fast and economic probe for the sensitive detection of SNPs in DNA.

Keywords: Bioanalytical, Biosensors, Luminescence, Nucleic Acids
A Compact Sensor for Sensitive Multi-Spectral Fluorescence Detection in Molecular Diagnostics

In molecular diagnostics, fluorescent probes are used for specific detection of genetic material associated with particular diseases, enabling physicians to diagnose diseases accurately and prescribe "personalized" treatments. Equipment for sensitive fluorescence detection are, however, typically expensive and bulky. Furthermore, additional optical elements are required to perform spectral separation when using multiple fluorescent probes for multiplex testing. The added optical elements inevitably reduce sensitivity, affordability and robustness of the system. A compact module for multi-spectral fluorescence detection can reduce the size, complexity and cost of fluorescence-based molecular diagnostic instruments. Capable of detecting below 1nM fluorescein, the low-cost multi-spectral fluorescence PixelSensor can help bring the power of molecular disease diagnostics to the point-of-care, in both the developed and developing worlds.

Keywords: Array Detectors, Biomedical, Biospectroscopy, Spectrophotometry
Application Code: Biomedical
Methodology Code: Sensors
In our preliminary studies, a new type of ion sensor was developed which contains a meta-stable merocyanine photoacid polymer that replaces traditional pH sensitive dyes (chromoionophores) and anionic sites (ion-exchangers) for ion detection. Currently, we have improved our previous ion sensor by incorporating a newer type of meta-stable photoacid compound. Similar to its predecessor, the membranes containing the new photoacid compound has shown to actively exchange protons with calcium ions upon activation with visible light, and the competitive ion-exchange process can be followed by UV-Vis spectroscopy. Herein, we have improved the response time, limit of detection, and dynamic range. Also, this ion sensor highly discriminates other cations such as Na+, K+, and Mg2+. With respect to these improvements, the experimental values highly correlate to the newly developed theory based on a coupled equilibrium involving the phase transfer of the analyte and H+ with accurate assumptions that could extend the traditional ion-selective optode theory. Further work is in progress to improve its complete reversibility, limit of detection, response time, stability, and extend the sensing mechanism to different cations by understanding the fundamentals involved during phase-transfer equilibria. Thus, these results are bringing us closer for designing a controlled and reversible with fast response time ion sensor based on a meta-stable photoacid.


Keywords: Characterization, Ion Exchange, Sensors, UV-VIS Absorbance/Luminescence
Application Code: General Interest
Methodology Code: Sensors
Non-covalently crosslinked molecular imprinted copolymers were prepared to recognize nitrophenol in an aqueous solution. Free radical polymerization was used to create random copolymers that primarily consisted of n-isopropylacrylamide as the backbone monomer with recognition and crosslinking comonomers. Molecular imprinting was carried out in both dioxane and dimethylsulfoxide. The recognition monomer that is used to create the binding site within the polymer is 4-vinylpyridine. Two different types of non-covalent crosslinks were used throughout the polymer randomly to maintain the binding site conformation. One type of crosslink involved excess base, vinylpyridine, interacting with an acid co-monomer, acrylic acid or methacrylic acid. The second type of non-covalent crosslink involved pi-stacking interactions between benzyl or naphthyl comonomers. Binding was studied by equilibrium dialysis. The results show that non-covalently crosslinked polymers selectively bind selected isomers of nitrophenol with high affinity.

ACKNOWLEDGEMENT: Partial support for this research was provided by NSF grant 1012897
An innovative ratiometric fluorescent indicator based on poly (N-isopropylacrylamide) (PNIPAM) responds to Cu(II) complexation. The polymer chain is divided into two portions. One portion is copolymerized with polyfluor 570: methacryloxyethyl thiocarbamoyl rhodamine B (Rhodamine-B), the donor fluorophore, and N-((4'-methyl-[2,2'-bipyridin]-4-yl)ethyl)-N-propylacrylamide (BiPy), the ligand which can complex Cu(II). The other portion is copolymerized with amine groups, which are functionalized with Alexa Fluor 647, the acceptor fluorophore. To improve the stability of the system, PNIPAM is grafted from a silica particle, the substrate. When temperature increases from 25°C, which is below the lower critical solution temperature (LCST) to 46°C which is above LCST, the acceptor/donor fluorescence emission ratio decreases slightly due to thermal quenching. The fluorescence emission of the fluorophores changes with addition of Cu(II) which bring positive charge to the backbone when binding with the ligands, causing the polymer chains to change conformation. That's because the efficiency of fluorescence resonance energy transfer (FRET) decreases with increased distance between the donor and acceptor. The ratio of donor and acceptor emission changes with different concentrations of Cu(II). At 25°C, when pCu=-log[Cu(II)] decreases from 7 to 4, the ratio decreases from 0.65 to 0.47. At 46°C, when pCu decreases from 7 to 4, the ratio decreases from 0.62 to 0.37. When another fluorophore pair fluorescein o-acrylate and Alexa Fluor 555 is adopted in place of rhodamine-b and Alexa Fluor 647, the ratio changes from 0.49 to 0.43 at both 25°C and 45°C. The system also works in wastewater effluent in that there is acceptor/donor ratio change upon addition of Cu(II). Therefore, the activity of Cu(II) can be sensed based on the efficiency change of FRET.

**Abstract Text**

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**Keywords:** Environmental/Water, Fluorescence, Metals, Sensors

**Application Code:** Environmental

**Methodology Code:** Sensors
Aerosil 200 silica nanoparticles are grafted to thermoresponsive poly(N-n-propylacrylamide) (PNnPAM) brushes, which are labeled with a fluorescence resonance energy transfer (FRET) donor, Polyfluor 570 Rhodamine B (Rhodamine), and an acceptor, Alexafluor 647 (647), via Reversible Addition-Fragmentation chain Transfer (RAFT) polymerization. The brushes are capable of selectively binding polar organics through molecular imprinting technology. Aerosil 200 silica nanoparticles can stay suspended in solution. Their reactive silanol groups make it possible to covalently conjugate the polymer brushes to the silica surface. RAFT is a form of living polymerization that allows us to locate the donor fluorophore in one block of the copolymer and the acceptor fluorophore on a second block, thereby generating P(NnPAM-co-Rhodamine)-b-P(NnPAM-co-647). PNnPAM brushes are thermoresponsive and they exhibit collapse in the broader temperature range of 5-22[degree]C after being imprinted. Our ratiometric indicator takes advantage of the thermal phase transition. We imprinted the copolymer at high temperature in aprotic organic solvent and then removed the template at low temperature in aqueous solution. After removal, the conformation of the copolymer changes when it selectively binds the analyte. This affects the extent of FRET between the donor and acceptor, leading to a change in acceptor to donor intensity ratio. Unlike traditional molecular imprinting technology based on covalent crosslinks, our binding site conformation is maintained by non-covalent acid-base and pi-pi stacking crosslinks. We observe selective binding of nitrophenol isomers with high affinity. This is confirmed by the fluorescence analysis and equilibrium dialysis. Our data suggest that our indicator can be applied to selective and sensitive sensing for polar metabolites and pharmaceutical compounds.

ACKNOWLEDGEMENT
This research was supported in part by NSF grant 1012897.
The identification and quantitation of adulterants in cocaine samples are important not only for toxicological and clinical perspectives, but also for forensic proposed related to determine the origin of the drugs, since the analysis of different samples of seizure can be useful for police intelligence purposes (traceability of drugs and drug dealers) [1]. Aminopyrine (AMP) has been widely used as an analgesic and antipyretic drug and as model substrate for in vitro and in vivo investigations of drug metabolism [2] and recently has been reported in the literature its identification as an adulterant in cocaine seized samples [3]. In this work, the electrochemical response of AMP was evaluated using three different working electrodes (gold, glassy carbon, and platinum). The platinum electrode was chosen as the working electrode material due to the best analytical signal in comparison with other bare electrode materials. The best conditions for the oxidation of AMP on platinum surface was studied and based on this conditions an analytical curve (Fig. 1) with linear range from 100 to 1000 µmol L-1 was obtained achieving a limit of detection of 30 µmol L-1. The sensor response was evaluated for detection of AMP in cocaine samples seized by Brazilian Police performing an AMP spike and the recovery value obtained was 105 ± 5% (n=3). The interference to other contaminants commonly found in this sample [1] was studied and no interference was observed indicating the accuracy of the proposed method. Financial support: FAPESP, CNPq and CAPES.

Fig. 1 – SWV recorded in a 0.1 mol L-1 phosphate buffer with platinum electrode. Parameters: step, 0.1 V and amplitude, 0.01 V. Inset: Analytical curve. Regression linear equation: Ip (µA) = -1.21 x 10-6 + 0.028 (CAMP/µmol L-1), R2 = 0.998.


Keywords: Analysis, Drugs, Forensic Chemistry, Voltammetry
Application Code: Homeland Security/Forensics
Methodology Code: Sensors
Photothermal therapy based on near infrared-absorbing nanoparticles has emerged as a promising treatment for cancer during the past decade. However, complete eradication of tumor cells is difficult due to the heterogeneous laser heating of the nanoparticles inside the tumor. Here, we developed a method to improve the efficacy of tumor treatment by combining photothermal with photodynamic therapy along with magnetic field drug delivery assistance using silicon 2,3-naphthalocyanine dihydroxide iron-oxide gold nanostars (SiNC-IO-Au NSts). SiNC was entrapped in the hydrophobic pocket of the polymeric monolayer on the surface of IO-Au NSts, which allows direct cellular delivery of the hydrophobic photosensitizer via the lipophilic plasma membrane. Raman spectroscopy was used to quantify the amount of SiNC delivered to the cells. Combined photothermal therapy and photodynamic therapy with SiNC-IO-Au NSts was shown to be far superior than the photothermal therapy or photodynamic therapy alone. It was confirmed that photothermal-photodynamic therapy with magnetic field assistance was a successful method in the eradication of head and neck cancer due to synergistic effects. SiNC-IO-Au NSt enabled photothermal-photodynamic therapy with magnetic field assistance has the potential of preventing tumor reoccurrence and may have an important impact on the treatment of head and neck cancer in the clinic.

Keywords: Bioanalytical, Light Scattering, Nanotechnology, Raman
Application Code: Nanotechnology
Methodology Code: Vibrational Spectroscopy
Nanocrystallite magnetic particles are attractive due to many important applications like magnetic drug delivery, hyperthermia for cancer treatment, ferro fluids, magnetic storage data etc. Nanosized particles of cobalt ferrite have been synthesized by an auto combustion method. The citrate – nitrate solution was prepared under molar ratio of 1:1. The final product has been received by self ignition then as burnt powder was calcined at 950 ºC. The formation and phase identification of the synthesized particles were confirmed by FTIR and XRD. The particle size was investigated by a Scanning Electron Microscope.

**Abstract Text**

Nanocrystallite magnetic particles are attractive due to many important applications like magnetic drug delivery, hyperthermia for cancer treatment, ferro fluids, magnetic storage data etc. Nanosized particles of cobalt ferrite have been synthesized by an auto combustion method. The citrate – nitrate solution was prepared under molar ratio of 1:1. The final product has been received by self ignition then as burnt powder was calcined at 950 ºC. The formation and phase identification of the synthesized particles were confirmed by FTIR and XRD. The particle size was investigated by a Scanning Electron Microscope.
Owing the importance of gold nanoparticles in catalysis, designing them has become a major focus of the researchers. Most of the current methods available for the synthesis of gold nanoparticles (GNPs) suffer from the challenges of polydispersity, stability and use of toxic and harmful chemicals. To overcome these limitations, we present a novel single step, biofriendly process for synthesis of fructose (monosaccharide), sucrose (disaccharide) and raffinose (trisaccharide) capped GNPs, wherein sugar is directly capped onto gold without the use of any secondary capping/stabilizing agent. Characterization of synthesized GNPs was done using various analytical techniques like transmission electron microscope (TEM), dynamic light scattering (DLS), SEM-EDS, and UV-Vis spectroscopy. The synthesized sugar GNPs (S-GNPs) were spherical in shape and in the size range of 15 ± 5 nm. p-Nitrophenol reduction assay was used to evaluate the catalytic reduction activity of various sugar capped GNPs. The effect of temperature and the size of ligand on catalytic activity were also evaluated at different temperature using UV-Vis spectrometer. Further, rate constant (k) was determined followed by its activation energy (Ea) and exponential (A) factor. Results of the study also helped to understand the relationship between sugar chain length (ligand) and catalytic activity. Given to the high activity and stability, S-GNPs might be useful as a catalyst for wide range of industrial and environmental applications. Results of these studies not only provide us a novel synthesis method but also yielded efficient catalytic agents. This research was support by KY NSF EPSCoR Grant 0814199, WKU research foundation.

Keywords: Characterization, Environmental, Nanotechnology, Spectroscopy

Application Code: Nanotechnology

Methodology Code: Other
As the leading cause of cancer deaths in the United States, lung cancer claims more lives each year than the next three most common cancers combined and has a 5 year survival rate of approximately 16%. This research explores the ability of small (<5nm diameter) gold nanoparticles presenting RGD-containing ligands to target $\nu_3$ integrin, a biomarker known to be overexpressed in activated endothelial cells and certain lung cancers. Variables investigated were the density of the peptides on the particle surface, the spatial confinement of the peptides by looping them back down onto the particle surface, the type of ligand used to passivate the particle, and the ability of the particles to target cells based on their $\nu_3$ integrin expression. This was accomplished through clonogenic assays using a $^{137}\text{Cs}$ irradiator and comparing nanoparticle treated samples with those untreated or treated with Cilengitide, c(RGDf(NMe)V), which is currently in clinical trials. Cell survival data fitted to the linear quadratic model was used to calculate dose enhancement factors and confirmed the ability of both Cilengitide and the peptide-conjugated nanoparticles to target cells with higher $\nu_3$ integrin expression. Although the peptide densities explored did not appear to impact the efficacy of the particles, significant differences were seen in the more spatially confined looped peptides, suggesting an increase in the binding affinity of the particles for the integrins. Finally, the particle passivating ligand, although not expected to play a role in the binding of the particle to the receptor, did impact the survival rate, and future studies will investigate this property.

**Keywords:** Bioanalytical, Nanotechnology, Peptides

**Application Code:** Nanotechnology

**Methodology Code:** Other
Redox enzyme-based reactions were applied for the synthesis of conducting polymer nanoparticles [1] and functional layers suitable for the design of electrochemical biosensors [2]. Glucose oxidase (GOx) E.C. 1.1.3.4. from Penicillium vitale was applied as biocatalyst, which is forming hydrogen peroxide that initiates polymerization of some conducting polymers. It was shown that this method is suitable for the synthesis of polypyrrole, polyaniline and some other conducting polymer based layers and nanoparticles. It was demonstrated that both dissolved and immobilized enzymes could be successfully applied for the enzymatic synthesis of conducting polymer-based nanoparticles. Here reported synthesis of nanostructures based on conducting polymers belongs to ‘green synthesis’ since except monomer required for the formation of conducting polymer any other environmentally dangerous materials are applied in this polymerization process. We also have demonstrated that formed nanostructures and nanoparticles shows good biocompatibility with living cells and when implanted in mice. We have demonstrated that during such kind of synthesis of nanoparticles and/or nanostructured layers the enzymes becomes entrapped within conducting polymer layer. In addition gold nanoparticles could be entrapped within formed conducting polymer layer.

Acknowledgement: This project was financially supported by Lithuanian Scientific Council. Project NanoZim’s, Nr. VP1-3.1-ŠMM-07-K-02-042.


Keywords: Chemically Modified Electrodes, Nanotechnology, Polymers & Plastics, Protein
Application Code: Nanotechnology
Methodology Code: Sensors
A biosensing platform based on metal nanoparticle surface plasmon enhanced fluorophore fluorescence was designed. It used a nanostructure of Ag@SiO2-DNA-fluorophore to selectively detect metal ions and small organic molecules. DNA hybridization occurred in the presence of the targets through the formation of base-target-base complex; accordingly, the fluorophore (Cy3) was brought to the surface of Ag@SiO2 core/shell nanostructures. Through controlling the thickness of the silica shell on the Ag nanoparticle, the fluorescence of Cy3 could be enhanced by the surface plasmon resonance of Ag nanoparticle for up to 2.5 folds. This metal-enhanced fluorescence (MEF) sensor provided high sensitivity and selectivity for the detection of Hg2+, Ag+, and coralyne. It may be developed as a new class of MEF biosensors for other metal ions and organic molecules analysis.

Keywords: Biosensors, Environmental, Fluorescence, Sensors
Application Code: Nanotechnology
Methodology Code: Fluorescence/Luminescence
The characterization, sizing, and quantification of metal-based nanoparticles (NP) in a variety of matrices using single particle-inductively coupled plasma-mass spectrometry (SP-ICP-MS) is becoming increasingly popular due to the sensitive nature of the technique. Nanoparticle events in the plasma are less than 0.5 ms in duration\(^{1}\); however, current quadrupole-based ICP-MS instruments are limited to instrument dwell times in the millisecond range and have data acquisition overhead that adversely affects data quality. Novel instrument settings and data processing techniques can be used to explore the benefits of continuous data acquisition rates as fast as \(10^{5}\) Hz (or 10 µs dwell times)\(^{2}\). This talk presents data on the different effects data acquisition rate has on the quality of data that can be obtained by SP-ICP-MS. The effect of varying the dwell time and its influence on particle integration, particle counting, particle sizing, and background signal is discussed. Data on identifying the significant instrument settings and their implications on nanoparticle characterization is also presented.


**Keywords:** Detection, ICP-MS, Nanotechnology, Spectrometer

**Application Code:** Nanotechnology

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
### Abstract Text

The remarkable performance of natural proteins motors have inspired scientists to create nanomotors capable of converting energy into movement and forces. Such nanoscale devices consist of a self-propelled structure equipped with sensing and/or actuating attachments (i.e. cargo loads or bio-recognition elements), and are capable of transporting cargoes in a rapid and controlled manner. Nanomotors are currently the subject of an intense interest due to their potential applications in nanomachinery, nanomedicine, fluidic systems, nanoscale transport and assembly, among others.

Nanomachines could offer innovative concepts in sensing and monitoring applications. The large cargo-towing force of modern micromachines, along with their ease of surface functionalization, can enable new target isolation (“Capture and Transport”) strategies. Self-propelled functionalized nanomachines offer considerable promise for the selective isolation of target analytes from untreated samples, thus obviating laborious sample preparation steps. This offers considerable promise for further miniaturization of lab-on-a-chip devices, including operation in nanochannels, and simplification of the powering requirements of such microchip systems. Catalytic micromotors have also shown to display a chemotactic behavior in the presence of a gradient of the fuel concentration, with a directed movement and increased speed toward higher peroxide concentrations. Changes in the swimming behavior in the presence of hazardous chemicals can offer direct and timely visualization of chemical stress. Here we will describe the use of such microengines nanoparticles for motion-based threat detection and the possibilities in the development of new lab on a chip systems based on active transport, for diverse environmental applications.

**Keywords:** Analysis, Environmental, Lab-on-a-Chip/Microfluidics, Nanotechnology

**Application Code:** Nanotechnology

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Currently green synthesis of silver and gold nanoparticles has generated a lot of interest in sustainable nanotechnology due to environmental implications. We have previously reported the synthesis of quercetin pentaphosphate (QPP) and quercetin sulfonic acid (QSA) using simple procedures and have characterized them with UV-vis spectroscopy, H1-NMR, 13C-NMR, P-NMR and LC-MS techniques1. The solubility of QPP was found to be 840 mg/mL and aqueous solutions of both QPP and QSA were stable for over a period of 1 year. We hereby report the green synthesis of silver and gold nanoparticles using QPP, QSA and other naturally-occurring flavonoids, Quercetin, Apigenin and their derivatives QPP and Apigenin Triphosphate (ATRP). The major obstacles to flavonoid applications are the poor solubility of most flavonoids in common organic solvents. Therefore the derivatized phosphate flavonoids such as QPP and ATRP which are very soluble in water served as a precursor for green synthesis of silver and gold nanoparticles. The synthesized nanoparticles were characterized using TEM, EDX, Uv-vis spectroscopy, HR-TEM and selected area electron diffraction (SAED). The TEM studies depict that the particles sizes range from 2-80nm. Formation of AgNPs and AuNPS were confirmed by EDS spectra. Crystallinity is confirmed by the SAED spectra and the fringes observed in HRTEM images and the results are in agreement with the UV resonance plasma peaks.


Keywords: Nanotechnology
Application Code: Nanotechnology
Methodology Code: Chemical Methods
The main aim of NANOMICEX project is to reduce the potential risk upon workers’ exposure to the engineered nanoparticles employed in the operative conditions of the inks and pigments industry. New surface modifiers are designed and developed to obtain less hazardous and more stable nanoparticles to achieve the goal. The work has focused on a set of nanoparticles such as metal oxides (MOs), quantum dots (QDs), silver nanoparticles (AgNPs) relevant to the ink and pigment industry. A complete characterization is conducted, followed by an exposure measurement in order to characterize and quantify any potential particle release in the production and processing activities. A comprehensive hazard assessment will allow the evaluation of effects on human and environmental models with comparisons between simple and modified nanoparticles carried out in the scope of the project. In this presentation, the relationship between the surface chemistry of metal oxide NMs such as ZnO, TiO2 and Al2O3, QDs and AgNPs versus toxicity will be the focus. A number of ligands and polymeric structures with biological origin are covalently attached to the NM surfaces and their cytotoxicity is evaluated. It is found that there is a strong relationship between surface chemical structure of NMs and their toxicity.

The authors acknowledge the financial support from European Commission and Yeditepe University.

Keywords: Environmental, Paint/Coatings, Particle Size and Distribution, Toxicology
Application Code: Nanotechnology
Methodology Code: Other
There is an enormous interest to use nanomaterials in widespread applications ranging from electronics and construction to medicine and consumer products. Although these materials provide major advantages regarding their unique physicochemical properties, a major concern has also been raised about nanomaterial toxicity to environmental and human health. Besides being time-consuming and expensive, the conventional techniques to determine nanotoxicity, however, have not been proved to be reliable at all conditions for all nanomaterials. Therefore, novel approaches are sought to evaluate nanotoxicity accurately. Among them, Raman Spectroscopy together with Surface Enhanced Raman Spectroscopy (SERS) are considered as alternatives to the conventional techniques. In this study, it is aimed to form a model to determine nanotoxicity with the help of Raman and SERS, followed by multivariate data analysis methods. Three model nanoparticles (single-walled carbon nanotubes, titanium dioxide and zinc oxide) are tested on two model cell lines (A549 and HDF). Additionally, for SERS studies, 13 nm gold nanoparticles are incubated with the cells. Raman and SERS are performed on living cells without any fixation procedure, bypassing the disadvantages such as fixative related peaks in the spectra and the change of cellular content distribution. The preliminary results indicate that it is possible to obtain a pattern from the multivariate data analysis, discriminating the nanoparticle treated and non-treated cells.

Acknowledgement
This project (Project code: 113Z554) is supported by TUBITAK and Yeditepe University.

Keywords: Data Analysis, Raman, Surface Enhanced Raman, Toxicology
Application Code: Nanotechnology
Methodology Code: Vibrational Spectroscopy
Comparison of Magnetic Properties of Synthesized Different Core-Shell Gold Nanoparticles

The superparamagnetic core/gold shell nanoparticles have attracted from many fields and applications such as protein and enzyme immobilization [1], bioseparation [2], drug delivery and immunoassays [3]. Three different magnetic gold nanoparticles synthesized in two steps via wet chemical methods in a various surfactants and stabilizers at room temperature. In the first step superparamagnetic Fe3O4 iron oxide nanoparticles prepared with alkaline aqueous media. After washing steps and protonation with HClO4, iron oxide nanoparticles used as magnetic core for the next step. Sodium borohydride and hydroxylamine hydrochloride used as reducing agent for gold shell formations on first and second nanoparticles. Seed-growth method with citrate stabilization was used for third nanoparticles. Transmission electron microscopy (TEM) images were obtained for three core-shell gold nanoparticles, results showed that two of gold nanoparticles were in spherical shape and third one is anisotropic shape with mean diameter of 20 nm, 15 nm and 23 nm respectively. X-ray diffraction (XRD) patterns indicated that the resulting iron oxide nanoparticles were pure Fe3O4 with spinel structure. Au patterns also observed after gold nanoparticles shell structure completed. Magnetic measurement by vibrating sample magnetometer (VSM) relieved that the saturated magnetization (Ms) of three god nanoparticles reached 50 emu/g, 37 emu/g, 27 emu/g and 56 emu/g respectively. All of the nanoparticles showed the characteristics of superparamagnetism. For magnetic nanoparticle synthesis, changes of size, shape and surfactants gave chance to adjust and control magnetic properties on nanoparticles. In this study we compare physical properties of three magnetic core-shell nanoparticles that were synthesized with different methods.


Keywords: Biosensors, Chemical, Materials Science, Nanotechnology

Application Code: Nanotechnology

Methodology Code: Physical Measurements
With the development of nanotechnology and the use of nanoparticles, the need to characterize nanoparticles has also grown. Many types of nanoparticles are now being used, with one of the most popular being silica dioxide. Due to its unique characteristics, SiO$_2$ nanoparticles are being used in a wide variety of application areas. For the most successful implementation, the size and size distribution of these particles must be characterized.

Silica measurement with ICP-MS is challenging due to the presence of $^{14}$N$^+$ and $^{12}$C$^{16}$O$^+$ which form in the plasma and have the same m/z as the most abundant Si isotope (m/z 28, 92%). As a result, the background at m/z 28 is very high, which inhibits low-level Si determination and could make detection of SiO$_2$ nanoparticles difficult. However, with Single Particle ICP-MS, the data acquisition rate is so fast, that the background is much lower than in conventional analyses.

This work will focus on the characterization of SiO$_2$ nanoparticles using SP-ICP-MS.

Keywords: Elemental Analysis, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Atomic Spectroscopy/Elemental Analysis
In the exploding field of nanoscience and nanotechnology, carbon nanotubes have attracted considerable attention owing to their unique mechanical and electronic properties for a broad field of potential applications.

SWCNTs are theoretically constructed by rolling up a graphene sheet into a cylinder with the hexagonal rings joining seamlessly. Depending on the way the graphene sheet is rolled up a huge diversity of SWCNT structures can be constructed differing in length, diameter and roll-up angle and with unique electronic properties, as they exhibit either metallic or semiconducting behavior.

UV/Vis/NIR absorption spectroscopy has proven to be a powerful tool in unmodified and processed SWCNT characterization, as a high information density related to the physical and electronic properties of the SWCNT sample is accessible by a readily available and inexpensive technique. Since various factors influence the optical properties of SWCNTs, absorption spectroscopy gives access to key information.

A suitable UV/Vis/NIR spectrophotometer needs to deal with high absorbing (black carbon) liquids as well as with low absorbance values with best possible signal to noise ratio and low spectral bandwidths in Vis and NIR. By an experimental comparison with SWCNTs it could be shown that the UV/Vis/NIR spectrometer Lambda 1050 WB from PerkinElmer showed the best performance available on the market and is the best choice for this kind of experiments. The Lambda 1050 offers a double monochromator system with very low stray light and allows to analyze samples up to 8 A (absorbance units) in Vis and NIR. With such a system, the sharp SWCNT peaks which are superimposed on the high absorbing background can still be precisely determined in the whole spectral region. The high signal/noise ratio also allows very low sample concentrations, e.g. down to 0.2 µg/mL.

Keywords: Materials Characterization, Nanotechnology, Spectrophotometry, Spectroscopy
Application Code: Nanotechnology
Methodology Code: UV/VIS
Nanoparticles have been growing in use in a large number of application areas. Cosmetic products such as sunscreens are one major application area. Nanoparticles are commonly used to enhance the protection from harmful ultra violet radiation by scattering the harmful radiation away from the skin. Characterization and quantitation of nanoparticles used in creams and lotions is a key element in understanding how homogeneously materials are distributed and the overall effectiveness of these nanoparticles. The unique use of UV/VIS spectroscopy coupled with a 150 mm integrating sphere and center mount sample holder provides important information to quantitate and compare the contributions of absorbance, large particle scattering, and nanoparticle scattering.

**Keywords:** Cosmetic, Materials Characterization, Nanotechnology, Spectroscopy

**Application Code:** Nanotechnology

**Methodology Code:** UV/VIS
Abstract Text

Single Particle ICP-MS (SP-ICP-MS) is a new operating mode in the world of ICP-MS dedicated to the analysis of metal based nanoparticles which allows the differentiation between ionic and particulate signals, quantitation both the ionic and particulate fractions, measurement of particle concentrations (particles/mL) and, particle sizes and exploration of agglomeration and size distribution.

This study outlines the quantitation and characterization of element oxide nanoparticles (Al2O3, and CeO2) commonly used in the nanoelectronics and semiconductor fabrication industry for the chemical-mechanical planarization (CMP) of semiconductor surfaces [1].

The characterization of the size distribution of CMP slurry nanoparticles, as well as the identification of larger particles, is an important aspect for the quality control of the photolithography process as they can impact the eminence of the silicon wafers. One of the most promising techniques for analyzing metallic nanoparticles is ICP-MS run in single particle mode (SP-ICP-MS). Due to its ability to measure both the dissolved concentrations of analytes and individual nanoparticles.

Keywords: Analysis, Characterization, ICP-MS, Semiconductor
Application Code: Nanotechnology
Methodology Code: Mass Spectrometry
Nanomaterials: Applications and Characterization

Rapid Analysis of Titanium Dioxide Nanoparticles in Sunscreen Using Single Particle Inductively Coupled Plasma-Mass Spectrometry Method

Titanium dioxide nanoparticles have been manufactured and used in many products. Most sunscreens contain different amounts of titanium dioxide nanoparticles. Single particle inductively coupled plasma-mass spectrometry (SP-ICP-MS) is an emerging methodology for nanoparticle characterization and quantification. In this study, SP-ICP-MS was used to determine the particle size, size distribution, particle concentration, and mass content of titanium dioxide nanoparticles in commercial sunscreens. A PerkinElmer NexION 300/350 ICP-MS with Syngistix™ Nano Application software the market only dedicated software for SP-ICP-MS was used for the high throughput analysis. Seven different brands of sunscreens containing different amount of titanium dioxide were tested. The sunscreens were dispersed in 1% Triton aqueous solution first, then further diluted with ultra-high purity water, and analyzed by SP-ICP-MS. From the SP-ICP-MS analysis, size, size distribution, particle concentration, and dissolved Ti were obtained simultaneously. The primary particle size detected spans from 35-45 nm for different sunscreens. Mass content of titanium dioxide in sunscreen was determined by standard addition method. Since the primary particle size detected is 35-45 nm, a 40 nm titanium dioxide nanoparticle was chosen as standard to be spiked into the sunscreen at different concentrations. The titanium dioxide content measured by the standard addition method is close to the manufacture claimed titanium dioxide content. The major advantages of the SP-ICP-MS analysis are fast analysis, informative results, and easy sample preparation.

Keywords: Cosmetic, ICP, ICP-MS, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Mass Spectrometry
The polymeric micro- and nanostructures are especially attractive for fluorimetric sensors preparation. This type of structures can be characterized by a wide concentration range of response and a relatively short response time.

In our work we proposed a novel type of nanospheres using alternating polymers, e.g. poly (maleic anhydride-alt-1-octadecene) or poly (styrene-co-maleic anhydride), as starting material. Simple synthesis approach yields nanospheres size ranging from about 120 nm to 250 nm. The spheres synthesized were used as fluorimetric ions - nanosensors using an optode approach. Thus obtained fluorimetric ion sensors where characterized by fast responses and were showing reproducible linear dependence of fluorimetric signal on change of logarithm of ions concentration in solution within the range from 10^-5 M to 10^-1 M (as receptor typical ionophore was used and chromoionophore as optical transducer). The unique linear dependence obtained was attributed to surface related phenomena on nanosphere/solution interface.

Keywords: Fluorescence, Luminescence, Sensors
Application Code: Nanotechnology
Methodology Code: Fluorescence/Luminescence
Heart disease is the number one cause of death in the US and among the group, cardiac arrhythmia is the most common in clinical practice. The current treatment for drug resistant cardiac arrhythmia is by catheter ablation. The catheter ablation uses radiofrequency or cryoenergy to ablate a selected region of the heart interrupting the fibrillatory conduction and finally terminating arrhythmia. However, this therapy is non-specific and recurrence of arrhythmia is often observed. Also, cellular damage to benign neighboring cells cannot be avoided leading to complications such as esophageal fistula and rupture. Thus a more cell specific targeting treatment is needed for a safer and more efficient way to treat arrhythmia. Here, we introduce a new, cell specific ablative strategy for treating arrhythmia through targeted photodynamic therapy (PDT), assisted by a tailor made photosensitizing nanoplatform. PDT is a clinically approved phototherapy, using light to excite a photosensitizer, so as to create reactive oxygen species (ROS) which, in turn, will kill the cells. PDT photosensitizers are fluorescent dyes that exhibit no toxicity in the dark but upon excitation with a certain wavelength of light create cytotoxic ROS that lead to cell death by either apoptosis or necrosis, depending on the light power. To a star shaped Poly-Ethylene-Glycol with 8 amine functional groups on the surface, a photosensitizer, Chlorin e6, and a targeting moiety, Cardiac Targeting Peptide (CTP), are conjugated. The size of the nanoplatform is properly controlled to be able to pass the cardiac capillary vessels’ fenestrations/pores whose size ranges from 6 nm to 25 nm. This photosensitizing nanoplatform has shown great selectivity toward cardiac myocytes, over other cardiac cells, and specific ablation of targeted cardiac myocytes is observed both in vitro and in vivo. In summary, a highly cell specific PDT assisted by nanoplatform can be an alternative novel therapy for cardiac arrhythmia.
Surface Analysis/Imaging

Complimentary Use of Raman and FTIR Imaging for the Analysis of Multi-Component Polymer Materials

Multi-layer polymer materials are highly engineered materials that are utilized in a wide variety of different industries and confirming the quality and composition of these materials as well as troubleshooting any structural failures is important to the manufacturers of the materials as well as the industries that use them. Both FTIR and Raman spectroscopy provide information on molecular structure and chemical environment. Both can be used for microscopic analysis of the thin layers associated with these polymer composites materials. However there are certain advantages unique to each of these spectroscopic methods.

FTIR provides excellent information on functional groups and polar constituents. Many functional groups found in polymers such as esters, hydroxyls, and amides produce very strong and diagnostic peaks in the FTIR. Raman spectroscopy is very sensitive to small changes in molecular structure and chemical environment and can give access to molecular backbone information that is less readily apparent in the FTIR spectra.

This presentation will compare and contrast these various micro-spectroscopic techniques for the analysis of layered polymer materials. This will include FTIR mapping and imaging examples using transmission, reflection, and ATR methods as well as Raman mapping and imaging examples including confocal depth profiling. The advantages and challenges of each of these methods will be highlighted and discussed.

Keywords: FTIR, Imaging, Polymers & Plastics, Raman
Application Code: Polymers and Plastics
Methodology Code: Surface Analysis/Imaging
Here we present a simple and fast optical transmission microscopy study on the wetting transitions on hierarchical polymer surfaces immersed in water. We analyze the influence of immersion time and the liquid pressure on wetting states of individual micro-cavities on these surfaces, as well as the lifespan of their superhydrophobicity. We show that transitions between the three wetting states (Cassie, Cassie-impregnating, and Wenzel) occur at a certain pressure threshold. Below this threshold, the transitions between the Cassie and the Cassie-impregnating states are reversible, while above the threshold, irreversible transitions to the Wenzel state start to occur. The transitions between the different wetting states can be explained by taking into account both the Young-Laplace equation for the water menisci in the cavities and the diffusion of dissolved gas molecules in the water. In addition, the wetting transitions had a stochastic nature, which may result from the diffusion of dissolved gas molecules in the water between neighboring cavities. Further, we compared the contact angle properties of two polymeric materials (COC and PP) with moderate hydrophobicity. We attributed the different water repellent properties of the two materials to a difference in the wetting of their nanostructures. The experimental observations indicate that both the diffusion of gas molecules in water, and the geometry of nanostructures influence the sustainability of superhydrophobicity of surfaces under water, understanding these factors can help improve the structural design of superhydrophobic surfaces.
Recently carbon nanotubes (CNTs) have been widely explored as materials for flexible, transparent, and conductive coatings for applications in optoelectronics. The advances of printing techniques have enabled the production of CNT electronics on flexible substrates or large-surface-area substrates. However, characterizing the printed CNT electronics is still challenging, especially for evaluating the print resolution, film homogeneity, and film thickness. Scanning electron microscopy (SEM) and atomic force microscopy (AFM) have been used to measure CNT film surface roughness and film thickness, but only a small number of samples can be analyzed, and the sample preparation and analysis are complex, which make these tests suitable only for laboratory studies.

Brewer Science has been working on printing and characterizing CNT electronic devices for applications including transducers, conductive traces, and high-speed device sensors. In this work, a simple and novel optical method has been developed for characterizing printed CNT electronics. The film homogeneity, surface roughness, and thickness of CNT electronics on different substrates were analyzed by using optical microscopy and profilometry. Two-dimensional (2-D) images of CNT electronics were captured by the optical microscope, and the average thicknesses of the CNT films were measured with the profilometer. By correlating the gray-scale or pseudo-color-scale distribution of the 2-D images with the thickness measurement results, the film thickness distribution and relative surface roughness can be obtained. This method provides a simple way for large-quantity sample analysis for printed CNT electronics within a short period of time. Moreover, it does not involve any complex sample preparation and thus can be used for analyzing large-surface samples. The detailed results and conclusions will be presented at the conference.

Keywords: Characterization, Imaging, Microscopy, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Surface Analysis/Imaging
Surface Analysis/Imaging

Magnetically Modulated Optical Nanoprobes (MagMOONs) for De-gelation Detection through Tissue

We describe a method to detect through tissue the chemically-induced changes in viscosity and de-gelation process of alginate gels using magnetically modulated optical nanoprobes (MagMOONs). The MagMOONs are fluorescent magnetic microspheres coated with a thin layer of opaque metal on one hemisphere. The metal layer prevents excitation and emission light from passing through one side of the MagMOONs, which creates orientation-dependent fluorescence intensity. The magnetic particles also align in an external magnetic field and give blinking signals when they rotate to follow an external modulated magnetic field. The blinking signals from these MagMOONs are distinguished from background autofluorescence and can be tracked on a single particle level in the absence of tissue, or for an ensemble average of particles blinking through tissue. This approach can potentially be employed in drug delivery systems based on enzyme-catalyzed breakdown of gel components.

Keywords: Biosensors, Detection, Imaging, Microscopy
Application Code: Bioanalytical
Methodology Code: Sensors
### Abstract Text
A component to understanding biological processes involves identifying the proteins expressed in cells as well as their modifications and the dynamics of processes. The technology to sequence proteins underwent significant disruption with the sequencing of genomes. Mass spectrometry has benefited from large-scale genome sequencing of organisms resulting in new methods for rapid and large-scale quantitative analysis of proteins. We’ve been developing mass spectrometry based methods for large-scale analysis of proteins in cells and recent developments will be discussed. Extrapolation of these analytical technologies to more complicated systems has allowed studies of brain function and disease. Application of these methods to the study of a depression model in rats will be described.

### Keywords:
- Mass Spectrometry, Proteomics, Software, Tandem Mass Spec
- Genomics, Proteomics and Other ‘Omic

### Methodology Code:
- Mass Spectrometry
Quantitative proteomics has made critical contributions to the determination of protein complex content and protein interaction networks. With the widespread use of quantitative proteomics, however, deeper insights into protein complexes can be obtained. Now, the tools exist to thoroughly analyze the dynamics of protein complexes when subjected to some form of manipulation. We have explored the use of deletion network analysis to determine the modularity of protein complexes and to accelerate the determination of uncharacterized protein function. To begin, little is known about how proteins interact and are organized within complexes. We developed a technique that combines biochemistry approaches, label-free quantitative proteomics and computational methods using wild-type and deletion strains to investigate the organization of interacting proteins within protein complexes. We have applied this approach to chromatin remodeling complexes and we find that we are able to experimentally determine modules within complexes. We continue to expand on these studies where we seek to determine the modularity in the entire yeast chromatin remodeling protein interaction network and we seek to define, analyze, and manipulate novel human protein complexes and protein interaction networks.

Keywords: Biological Samples, Mass Spectrometry, Protein, Proteomics
Application Code: Genomics, Proteomics and Other 'Omins
Methodology Code: Mass Spectrometry
Proteomics technology has advanced at a breathtaking rate since the development of soft-ionization techniques for mass spectrometry-based molecular identification over two decades ago. Now, the large-scale analysis of proteins (proteomics) is a mainstay in nearly all aspects of biological research including clinical research, where there is a high premium placed on molecular diagnostics and personalized medicine. While peptide-based proteomic strategies (bottom-up proteomics) were developed and optimized early and represent the gold-standard at present, we are now beginning to understand the limitations to bottom-up technology, namely the inability to characterize and quantify intact protein molecules from a complex mixture of digested peptides. To overcome these limitations, numerous labs have taken a whole protein-based approach to proteome research where intact protein molecules are the subject of analysis and full characterization and quantification can be facilitated. A discussion of these ‘top-down’ techniques and how they have been, and will likely be, applied to biological research of clinical relevance is the topic of this talk. With the unprecedented precision offered by mass spectrometry-based proteomics, both peptide- and whole protein-based strategies are poised to be used as complementary techniques to characterize complex disease phenotypes in the 21st century.
The use of quantitative proteomic approaches within studies of virus-host interactions during infection provides a powerful mean for defining cellular defense mechanisms and for discovering new targets of antiviral therapeutics. By integrating quantitative mass spectrometry with molecular virology, microscopy, immunoaffinity capture, and bioinformatics, we characterized human proteins that regulate immune responses following viral infection.

In mammalian cells, the first line of defense against viruses involves sensing of pathogenic nucleic acids, such as viral DNA. The recognition of viral DNA is accomplished by specialized proteins, called sensors, which can bind to viral DNA and trigger intrinsic and innate immune responses. The human interferon-inducible PYHIN proteins (AIM2, IFI16, IFIX, and MNDA) have emerged in recent years as critical regulators of immune response. We have characterized the antiviral functions of IFI16, the first host factor shown to detect viral DNA in nuclei of infected cells. As viruses have acquired effective mechanisms for blocking host immune signaling, we went on to define the virus-host interactions aimed at blocking IFI16 defense functions during infection with several herpesviruses. Furthermore, we constructed the interaction network for all PYHIN proteins, and this presentation will cover strategies for quantifying protein interactions, building functional interaction networks, and determining the roles of posttranslational modifications during infection. Based on the association of PYHIN proteins with other antiviral factors, we go on to characterize another previously unrecognized DNA sensor important for mounting immune responses to herpesvirus infection. Our studies underscore the value of integrative proteomics in deducing protein function based on interaction networks, as well as characterizing important immune functions mediated by human PYHIN proteins.
Analyzing the metabolome is made difficult by the extreme complexity of samples, disparate polarities of metabolites, and large range of analyte concentrations. Sample preparation must also be considered carefully because metabolite concentrations can change rapidly. LC-MS has proven to be a valuable tool for metabolomic analysis. Polar metabolites can be challenging to determine because sensitivity and reproducibility of HILIC may not be sufficiently high, but retention on reversed phase columns is too low. For polar metabolites, we have investigated derivatization using benzoyl chloride and aniline. Benzoyl chloride labels amines and phenols and aniline labels carbonyls and phosphates. These reagents enhance retention and sensitivity substantially for reversed phase LC. Further, because 13C-labeled forms are available, stable isotope internal standards can be made for every analyte. Because of the broad reactivity of these reagents, they cover a large fraction of the polar metabolome. Using this approach has proven especially valuable for metabolite profiling where specific pathways are targeted. For example, an assay that monitors over 70 neurochemicals has been developed. This assay has been coupled with microdialysis to monitor in vivo neurochemical changes during behavior of different phenotypes of rats. This and other assays based on the technology has also proven useful for characterizing pathways in insulin secreting cells, plasma, and fruit flies.

**Keywords:** Biological Samples, HPLC, Metabolomics, Metabonomics

**Application Code:** Neurochemistry

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
This presentation will cover work in three areas in which the author has been mainly involved. They are mid-FT-IR (and Raman), Near-IR (NIR), and Thermal Analysis. The mid-FT-IR applications will include trace level moisture determination in electronic gases, determination of sulfur oxide species in white liquor, and identification of ethylene core shell surrounding propylene spherical particles and determination of its thickness. The on-line mid-FT-IR, developed by using a system from Remspec, will discuss its use as a process R&D tool. The NIR projects, carried out in quality control and in process control, will demonstrate the unique benefits of NIR: minimal sample preparation, simultaneous determination of multiple components, no need for solvents and subsequent waste disposal, safer operations, time and cost savings, and automatic process control. Work on biomolecules carried out as a Humboldt Fellow at the University of Munich and the Max Planck Institute will be discussed next. It will conclude with thermo-gravimetric analysis (TGA) work carried out in collaboration with several co-op students by developing the finger print concept from FT-IR and fractional distillation from physical chemistry. The above topics will be gratefully interspersed with names of colleagues, mentors, and friends with whom the author has had the privilege of working.

Keywords: Characterization, Materials Science, Method Development, Vibrational Spectroscopy
Application Code: Polymers and Plastics
Methodology Code: Molecular Spectroscopy
When a new technology emerges, early adopters like Jagdeesh Bandekar play an important role in defining the direction it will take. Over the last 25 years, the use of in-situ spectroscopic methods to monitor chemical and biochemical reactions has become almost a routine approach to chemical research and product development. This talk will highlight some of the major steps along the way, and describe some varied examples of the adoption of real-time, in-situ monitoring methods with applications as varied as restoring medieval wall paintings and cleaning studies in the pharmaceutical industry, to academic and industrial studies of reaction chemistry.
Raman spectroscopy is a nondestructive technique capable of providing information at the molecular level. In the present work we have investigated the cellular regions in neuroblastoma and ganglioneuroma using Raman spectroscopy, and compared their spectral characteristics with those of the corresponding normal adrenal gland. Raman spectra of frozen and paraffin preserved tissues were recorded and analyzed in conjunction with the pathological examination of the tissues. The spectral analyses of the data shows that the normal adrenal gland tissues have higher levels of carotenoids, lipids, and cholesterol compared to the neuroblastoma and ganglioneuroma frozen tissues. A comparison of Raman spectra of paraffin preserved and frozen tissues reveals that preserving the tissues in paraffin alters the molecular composition of the tissues, and in particular, entirely removes carotenoids, lipids, and cholesterol in the adrenal tissues. The Raman spectroscopic data has been quantitatively analyzed using chemometric methods of principal component analysis and discriminant function analysis, and the analyses show that Raman spectra can clearly classify the tissues into their pathological groups with high sensitivity and specificity.
Laboratory data show that adhesive bonding (particularly along with spot welding) is an effective and inexpensive way for light-weight-vehicle designs. However, since the early ‘90s, the general consensus among automakers, steelmakers, and adhesive suppliers is that bonding Zn-Fe hot-dip coated (Galvannealed) sheet steels using adhesives is catastrophic and infeasible. In 2007, a collaborative joint project between Dow Automotive and ArcelorMittal R&D was initiated to address the feasibility of using adhesive bonding on galvannealed steel panels for automotive body applications. The common unfavorable conception of adhesive bonding on galvannealed steel is identified as resulting mainly from erroneous judgment in galvannealed steel grade selection. Most previous adhesive bonding data were based upon results using Galvannealed Interstitial Free (GAIF) steel, which is primarily used for exposed car body applications, not for structural applications. The clean steel grain boundary of IF steel makes it highly formable but also highly susceptible to liquid Zn penetration/embrittlement that leads to weak steel/coating interface. On the contrary, Advanced High Strength Steels (AHSS) that are used primarily for structural applications display satisfactory adhesive bonding results due to consistently stronger interface strength. In this presentation, positive results from a series of adhesive bonding tests on different grades of GA AHSS will be presented along with residual stress measurements by X-ray diffraction to reveal the degree of liquid Zn penetration in the steel grain boundary.

Keywords: Materials Science, Metals, Microscopy, X-ray Diffraction
Application Code: Materials Science
Methodology Code: X-ray Techniques
### Abstract Text

Rheology is the study of the flow and deformation of matter. In flow testing, one measures the viscous properties of a fluid material; in deformation testing, one obtains knowledge of a solid-like material’s strength and elasticity. In actuality, the most common usage of commercial rheometers is to determine a material’s viscoelastic properties since most materials fall within the extremes of being completely fluid-like and completely solid. The most common instrument for performing rheological measurements is the rotational rheometer. Part of the talk will contain rheology basics as they pertain to rotational rheometry. The development of the rotational rheometer from a historical perspective will also be discussed. One of the key classes of tests performed with the rheometer is dynamic oscillatory testing. This is the best way of measuring a material’s viscoelasticity. Different dynamic methods will be discussed, and examples will be shown how rheological testing has resolved real-life applications issues. Some novel methods that are gaining popularity, such as Large Amplitude Oscillatory Strain, will be included.

### Keywords:
- Rheology

### Application Code:
- General Interest

### Methodology Code:
- Other
The overall goal of this USDA-ARS research is to promote and retain access of United States-grown crops to domestic and foreign markets through compliance with maximum residue level (MRL) regulations. General research includes: objective 1) the development and validation of methods for residue analysis (i.e., How do we detect the residues? – as related to having confidence in any MRL), objective 2) the characterization of residues across a commodity, use patterns, and geography (i.e., How do the residues levels vary? – as related to an appropriate “ceiling” for an MRL), objective 3) tracing the formation and elimination of residues through production and marketing channels (i.e., How long do the residues last? – as related to specific contributions to the MRL), and objective 4) acquiring residue data from field trials following the application of a registered agrochemical (i.e., What is the expected maximum residue level? – as related to establishing an MRL tolerance in EU). Recent research findings will be presented and discussed.

Keywords: Agricultural, Environmental/Water, Liquid Chromatography/Mass Spectroscopy, Pesticides
Application Code: Agriculture
Methodology Code: Liquid Chromatography/Mass Spectrometry
The movement of cargo in international trade has resulted in introduction of numerous exotic insect pests and pathogens into the USA. Collectively, these organisms cause billions of dollars annually in crop losses, damage to natural resources, control measures and eradication programs. Early detection is essential in preventing introductions and facilitating eradication efforts when introductions occur. Currently, USDA Animal and Plant Health Inspection Service and DHS Customs and Border Protection rely primarily on visual inspection to detect exotic pests. These measures are expensive and often fail to find pests associated with commodities and packing material. Advances in chemical detection capability have improved the ability to detect pests based on their unique chemical signatures. We report on the development of a versatile sensing platform using chemosensitive colorants for detecting characteristic volatile compounds associated with several insect pests of quarantine significance in the USA. This sensing platform may enable the detection and identification of infestations based on VOC signatures within a closed shipping container during transit. This would allow infested containers to be identified and rejected prior to arrival, thereby mitigating exposure or release of quarantine insects. These devices can thus protect the shipments themselves, as well as agricultural commodities and natural resources in the USA.

Keywords: Analysis, Detection, Headspace, Volatile Organic Compounds
Application Code: Agriculture
Methodology Code: Sensors
As the trade of foodstuffs increases across the globe, preserving and protecting our commodities from pests is ever important. Postharvest fumigation is a valuable tool for achieving pest-free security and must be monitored to ensure its success. For example, measurement of the amount of fumigant that remains in the chamber over the course of fumigation is critical. Gas chromatography (GC) is frequently relied upon by regulators to verify the concentration of fumigant during phytosanitary treatment subject to international approval. Unfortunately, gas chromatographs must be outfitted with detectors respective to the fumigant. Therefore, many detectors are required in a laboratory to conduct the requisite analyses. Moreover, the operation of each GC detector typically requires the use of at least three different gases Helium (He), Nitrogen (N2), Oxygen (O2), and/or Hydrogen (H2). The Shimadzu Tracera GC outfitted with a barrier discharge ionization detector (BID) overcomes this need for multiple detectors as well as the need for multiple operational gases. The BID, which operates with He only, is capable of detecting nearly every contemporary fumigant, including: methyl bromide (MeBr), phosphine (PH3), propylene oxide (PPO), sulfuryl fluoride (SF), and ethyl formate (EF). In addition, physiologically-active gases can be detected via BID such as carbon dioxide (CO2), carbon monoxide (CO), and Oxygen (O). This allows us to monitor critical aspects of a postharvest fumigation treatment to identify whether or not physiologically-active gases are influencing the efficacy of treatment. We compared the limits of quantification (LoQs) for commonly used GC detectors, including, flame ionization detector (FID), pulse flame photometric detector (PFPD), and BID respective to MeBr, PH3, PPO, SF, and EF fumigants.

Keywords: Agricultural, Gas Chromatography, GC Detectors, Quantitative
Application Code: Agriculture
Methodology Code: Gas Chromatography
California industry recently learned of residue reports, from several officially recognized analytical laboratories within the European Union (EU), which indicate certain loads of tree nuts from California were not compliant with maximum residue level (MRL) tolerances set by the EU for the fungicide, fosetyl-aluminum. A review of pesticide use data collected by California Department of Pesticide Regulation (CDPR) does not support a contribution of fosetyl-aluminum to the MRL exceedance. The residue reports from the EU corroborate the usage data from the CDPR report, as all exceedances were attributed only to the detection of phosphorous, the degradation product of fosetyl-aluminum, with no concomitant detection of fosetyl-aluminum itself. Analyses conducted by DFA of California, in cooperation with USDA/ARS, failed to detect any fosetyl-aluminum in tree nuts, but did detect phosphorous acid. These tests also uncovered issues with the EU methods of analysis, indicating that residues of phosphoric acid could be mistaken for phosphorous acid. We report the development of LC-MS methodology that yields unequivocal resolution of phosphorous and phosphoric acid in tree nut samples.
Increasing import/export of foodstuffs in the international agricultural marketplace has led to increasing demand for fumigation treatments to insure that a given commodity is free of invasive species and diseases. With any new treatment, however, comes the need to quantitatively determine the residue of the fumigant remaining in the treated commodity. Fumigant residues are a potential trade barrier if the residue is in excess of what the receiving country has deemed permissible, a tolerance known as the maximum residue limit (MRL). Since fumigants generally range from highly volatile compounds, such as propylene oxide (PPO), to permanent gasses, such as methyl bromide (MB); residue levels are commonly determined by blending the commodity in the presence of solvent, letting the fumigant reach equilibrium between the solid, liquid, and gas phases and measuring the fumigants headspace concentration. These methods are effective, but are also time-consuming, wasteful, and require carefully controlled extraction conditions. We present alternate methods for the determination of PPO and MB residues in multiple commodities using a headspace trap autosampler (Perkin Elmer, Turbomatrix) or solid phase microextraction (SPME). Using the Turbomatrix allows for the detection of PPO, as well as its toxic degradation products propylene chloro/bromo hydrin, at levels ~3 orders of magnitude lower than those observed with liquid extractions (60ng/g vs. 30µg/g). Analysis of MB residues using either the Turbomatrix or SPME sampling allows for sub-ppm levels of detection with a quick one-step, solvent-free preparation method, rather than extraction with a buffered solution, followed by several hours of equilibration.

Keywords: Agricultural, Gas Chromatography/Mass Spectrometry, Headspace, SPME
Application Code: Agriculture
Methodology Code: Sampling and Sample Preparation
Analytical Overview of Agribusiness Demand in Brazil

Overview of agribusiness in Brazil. Veterinary Drugs, Mycotoxins and Pesticides, Overview on legal aspects and requirements in BR. International agreements, normative instructions (regulations), Ministry Guidance, Quality Control Procedures. Overview about industry, consumer and producer (what is being done, history). Main methods and norms: 2002/657/EC; SANCO/825/00 (pushes towards multiresidue methods for enforcement), QuEChERS, ChemElut, Sweet, DFG S19, Sampling. Brazilian government programs: Results of PARA (ANVISA), PNCR (MAPA) and relevant information. Challenge and tasks from retailer – Grupo Pão de Açúcar, Carrefour, Walmart no Brasil. Brazilian laboratories and sophisticated equipment and techniques. Accreditation by INMETRO standards in analytical quality ISO IEC 17025 and promising market to reference material.

Keywords: Analysis, Calibration, Food Contaminants, Food Safety

Application Code: Agriculture
Methodology Code: Chemical Methods
**Session Title**: Brazil - Analytical Chemistry in Agrobusiness  
**Abstract Title**: Rapid and Non-Invasive Time Domain Nuclear Magnetic Resonance Methods for Analyses of Agri-Food Products

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**Abstract Text**

High-resolution Nuclear Magnetic Resonance spectroscopy (NMR) has been used in qualitative and quantitative for 60 years. Nowadays, this analyses uses expensive spectrometers based on high field superconducting magnets. Another class of NMR spectrometers based on cheap permanent magnets, known as time-domain NMR (TD-NMR) has been successfully used as a laboratory technique for quality control (QC) and quality assurance (QA) for more than 40 years. Nowadays, TD-NMR is an off-the-shelf NMR solution that is recognized as a standard international method for several agencies. Despite its recognized success in the laboratory application, it has not been widely used in non-invasive analysis of agrifood products in packing houses or factory floors. The major limitation has been the dimension of magnet/probe, to fit high volumes samples (~1L). The recent developments of TD-NMR industrial sensors, based on yokeless 0.23 T Halbach permanent magnet for 1 L sample, for atline and inline analyses of fresh fruits, meatand industrialized food products, direct in the packages, will be presented.

**Keywords**: Agricultural, Food Science, NMR, Quality Control  
**Application Code**: Food Science  
**Methodology Code**: Magnetic Resonance
Brazil has the largest market for pesticides in the world, and has the greatest potential for expansion. To avoid the risks of pollution the fountains, soil and damage to human health, 94% of the total number of primary plastic containers for agrochemical products sold in the country are disposed after being returned by farmers at more than 400 receiving units. Through this initiative, Brazil is a world reference in the correct final disposal of packaging phytosanitary measures, ahead of countries like Germany, France, Japan and United States. Only plastic containers rinsed after use can be recycled, this procedure removes more than 99.9% of the active ingredients, turning the packaging of pesticide, once considered special waste in common wastes. To ensure that the packages were washed according to the right procedures and that is not occurring contamination of soil and groundwater in units of receipt there is a residue monitoring program. In the laboratory, the plastic packaging are washed inside with deionized water. Water rinse residues has extracted by liquid/liquid partition or SPE and quantified by GC/MS, or injected directly in the LC/MS/MS. The concentration of pesticides must be less than 100 mg/L. For soil and groundwater the residues are extracted by ultrasonic and liquid/liquid partition, respectively, and quantification performed by GC/MS and LC/MS/MS.
A few decades ago there were practically no pesticide residue analysis in Brazil. The main reasons were the lack of appropriate official food, health and agricultural surveillance legislation and qualified technical people. Nowadays, it is quite different: there are several laboratories with qualified staff and good facilities to perform pesticide analysis at international standards.

In the last fifteen years the Institute of Technology of Pernambuco (ITEP) has been supporting the fruit production through the analysis of pesticides residues. It is an Institute with the mission of providing technological support to Brazilian Agribusiness and Food Safety. The LabTox/ITEP is accredited under ISO/IEC 17025 by the Brazilian accreditation board, INMETRO, a member of the International Laboratory Accreditation Cooperation (ILAC) and signatory to the International on Mutual Recognition Agreement.

With an adequate infrastructure detecting more than 500 compounds by mass spectrometry techniques (LC-MS/MS and GC-MS/MS) besides well trained MSc and PhD staff, LabTox / ITEP has an innovative and competitive role to support the food safety and fruit quality export programs.

In order to expand its action, LabTox/ITEP introduced a monitoring and inspection fruit program overseas; this program started in 2010 and performs as if it is the outside "eye" of the producer. It is an important tool for production, packing and shipping improvements.

Despite the late start and typical difficulties intrinsic of developing countries, Brazilian pesticide analysis laboratories are now proficient to support the agribusiness and, most important, protect the health and the environment.

Keywords: Analysis, Chromatography, Mass Spectrometry, Pesticides
Application Code: Agriculture
Methodology Code: Mass Spectrometry
Strong coupling between the cell membrane and the recording electrodes is crucial for sensitive measurement of cellular electrical activities. We are interested in exploring nanotechnology and novel materials to improve the membrane-electrode coupling efficiency. The first approach involves developing vertical nanopillar electrode made of noble metals. The nanopillar electrodes deform plasma membrane inwards and induce negative curvature when the cell engulfs them, leading to a reduction of the membrane-electrode gap distance and a higher sealing resistance. The 3D topology of the nanopillar electrodes is crucial for its enhanced signal detection. The second approach explores a nanoelectrode of a new topology, namely nanotubes with hollow centers. The nanotube geometry further enhances membrane-electrode coupling efficiency and records larger intracellular potentials than nanopillar electrodes. The nanotube topology also significantly increases the time duration of intracellular access. Interestingly, the presence of high membrane curvature induced by vertical nanostructures, affects protein distributions and induces accumulation of certain proteins around them. Those results show a strong interplay between biological cells and nanosized electrode, which is an essential consideration for future development of interfacing devices.

Keywords: Electrochemistry, Electrode Surfaces, Electrodes
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Insects have been used extensively used as a model organism in genetics research and has significantly contributed to understanding molecular, cellular and evolutionary aspects of human behavior. However, chemical measurements of these mass-limited samples are still challenging. Research has focused on developing methods to obtain highly sensitive chemical quantification along with spatiotemporal information. The goal of chemical analysis is to relate neurochemical changes to either genetic mutations or to treatments that alter the state of the organism. Here, we develop methodology to rapidly quantify neurotransmitter and metabolite molecules. Chemical derivitization of biological molecules coupled with mass spectrometric detection will be used to measure neurotransmitter concentrations. The methods presented are broadly applicable and can be used with a wide range of analytes. Additionally, this scheme simplifies detection and makes rapid relative quantification possible, allowing comprehensive and rapid analysis of neurotransmitters. This enables the analysis of attomole amounts of neurotransmitters and metabolites. We investigated changes at individual cultured neurons from drosophila.

Keywords: Bioanalytical, Mass Spectrometry, Neurochemistry, Small Samples
Application Code: Neurochemistry
Methodology Code: Mass Spectrometry
Chemical Analysis in Super-Small Living Systems

Analysis of Homolymph from Individual D. Melanogaster: From Small Molecules to Proteins

Drosophila melanogaster or fruit fly has been a popular model in biological research and the study of human disease. The ease of genetic manipulation, a completely sequenced genome and a high degree of homology with the human genome are key factors in the continued popularity of the fly as a model system. The small size of the fly complicates its use as a model as the volumes available for analysis range from a couple hundred to tens of nanoliters from larval and adult flies. Method are described in this presentation to collect fly hemolymph or blood from individual flies for analysis. The determination of the chemical content of individual fly hemolymph requires the use of small volume compatible methods such as capillary electrophoresis (CE). CE has been used to quantify amino acid content in both larval and adult flies and has been performed to characterize the population distribution of various constituent amino acids. In these studies it is also possible to sample twice from an adult fly to study the effects of an experimental perturbation to an individual subject. Also, assay methods were improved to characterize thiol-containing amino acids and peptides in a study of oxidative stress with individual flies. This assay in conjunction with earlier amino acid analysis provided a means to explore the physiological effects of a transporter that affects basal glutamate levels in the fly. The characterization of the proteome of fly hemolymph has also been demonstrated with these exceedingly low volume samples. Proteins, predicted by genome or only observed at the transcription level, are reported using bottom-up proteomics. Novel fractionation methods were developed to aid in this characterization of hemolymph. New methods for separating hemocyte content from hemolymph allow the determination of amino acids solely from hemolymph plasma. The impact of the method of hemolymph collection including any anesthesia on observed amino acid content will be discussed. The methods of hemolymph analysis provide a look into this exceedingly small but important model for to understand behavior and disease.

Keywords: Amino Acids, Capillary Electrophoresis, Proteomics, Small Samples

Application Code: Bioanalytical

Methodology Code: Capillary Electrophoresis
Chemical Analysis in Super-Small Living Systems

Genetic Control of Neurotransmission in Drosophila

Drosophila melanogaster is a powerful model organism due to its simple maintenance, short life cycle, and impressive array of genetic tools. Our lab has pioneered using Drosophila optogenetics to evoke monoamine neurotransmitter release in combination with real time detection by fast-scan cyclic voltammetry. Channelrhodopsin-2, a blue light-activated cation channel, is expressed in neuronal subpopulations using the Gal4/UAS genetic system. Recent studies with pulsed stimulations have shown that serotonin and dopamine release are steady state at low frequencies and peak shaped at high frequencies. Uptake inhibition with fluoxetine removes the frequency dependence of release, proving uptake serves to regulate these extracellular concentrations. Recently, we have also used a new red-light activated channel, CsChrimson, for optogenetic control of larval neurons in discrete brain regions. Using CsChrimson, the kinetics of dopamine uptake and release are compared in the larval ventral nerve cord (VNC) and the protocerebrum. Vmax and Km are both an order of magnitude smaller in the protocerebrum than the VNC. Finally, we have used an ATP activated channel, P2X2, to examine the regulation of releasable pools of dopamine, which are regulated by both uptake and release. Unlike mammals, Drosophila do not have an additional storage pool that can be activated by cocaine. By using these new methods of neurotransmitter release to investigate the fundamental regulation of amine neurotransmission in the fly brain, we will gain a more thorough understanding of the role of monoamines in behavior and pathology.

Funded by NIH R01 MH085159 and Biogen Idec, Inc.

Keywords: Electrochemistry, Electrodes, Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
We have used in vivo voltammetry in the fruit fly, Drosophila melanogaster, to examine the competitive effects of methylphenidate on the actions of cocaine. Oral consumption of methyl-phenidate sufficiently blocks the Drosophila dopamine transporter and the blockage is concentration dependent. For untreated flies the rate of clearance changes 40% when the dopamine transporter is blocked with cocaine, for treated flies the number is 110%. In collaboration with David Krantz at UCLA, we have also taken the challenge to electrochemically measure neurotransmitter release from the smallest varicosities of a nerve cell. The nerve terminals found in the body wall of Drosophila larvae are readily accessible to experimental manipulation. We report the development of amperometry to measure release of the neurotransmitter octopamine from individual varicosities in the Drosophila larval system. Octopamine release was measured in the type II varicosities of Drosophila larvae. When octopamine is released from the varicosities during stimulation, it is immediately oxidized at a carbon fiber electrode and a current spike results. This provides an exciting new analytical method to study release at subcellular regions along neurons. Finally, we use mass spectrometry, both as a detector and for imaging, to determine the doses of methylphenidate when administered and to image this in the fly brain. We have discovered via imaging that many of the basic phospholipid species in the brain also vary, and spatially, in the brain following administration of the drug.

**Keywords:** Bioanalytical, Electrochemistry, Electrodes, Mass Spectrometry

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
For the last eight years, we have been using bioinformatics and MS based approaches to characterize HIV-1 vaccine candidates containing the surface envelope glycoprotein, Env. This protein is extensively glycosylated: it contains up to 30 different N-linked glycosylation sites and at least two potential O-linked sites. We have focused on characterizing the glycans at each of these sites using glycopeptide analysis strategies. By profiling a variety of different forms of the protein, we have answered several questions of high importance to vaccine developers, including: What is the glycan profile of the most native form of Env, that of a trimeric protein isolated from a membrane surface? Is there a unique glycosylation profile that is observed on more effective vaccine candidates? This presentation will highlight some of our most recent work in answering these questions, and we will use our Env analyses as an example of how mass spectrometry is a critical component in decoding the biological roles of glycans.
Decoding the Biological Roles of Glycans through MS and Tandem MS

Glycomics

The developments of analytical methods for proteins have produced highly effective tools for identification that are sensitive and comprehensive. The vast majority of proteins are post-translationally modified, however proteomics methods have not advanced as rapidly in this area. The most complicated post-translational modification is also one of the most common. Glycans are composed of up to 20 monosaccharide units as glyconjugates in proteins and lipids. Glycosylation is a modification where the structures are more complicated than that of the proteins. While sequences define the polypeptides, glycans are complicated by having many linkages, branching, and stereomeric residues. Understanding the roles of glycans depends on the ability to identify and quantitate hundreds of structures simultaneously. Research in our laboratory has focused on the rapid methods for the analysis of protein and lipid glycosylation while employing mass spectrometry and advanced liquid chromatography. These methods are employed in determining biomarkers for diseases including cancer and autoimmunity. They are also used for monitoring health by determining the biologically active components in mammalian milk.

Keywords: Liquid Chromatography, Mass Spectrometry, Time of Flight MS
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
The influenza A virus (IAV) which is the primary causative agent for flu epidemics initiates infection by binding to airway epithelia through a coat surface glycoprotein called Hemagglutinin (HA). HA displays N-glycosylation at between 5 and 12 sites, depending on strain, which change rapidly to maintain infectivity and survive selection pressures from exposure to the host immune system. Limited knowledge on these viral coat protein glycosylation sites and glycosylation patterns is available, based mostly on the information about N-glycosylation sequons (NxS/T) from genetic databases.

A multidimensional analysis workflow, yielding complementary information on the glycoproteins, was used to produce results with an acceptable confidence level. Recombinant HA1 samples from commercial sources were subjected to bottom-up LC-MS analysis. Proteolytic glycopeptides were analyzed using reversed-phase LC-MS/MS coupled with online HILIC (Hydrophilic interaction liquid chromatography) enrichment to get site-specific glycosylation information. Peptides deglycosylated in the presence of H218O were subjected to a standard proteomics analysis combined with database searching to reveal the glycosylation sites. Released N-glycans were subjected to negative mode glycomics analysis using HILIC LC-MS.

Correlating the patterns of glycosylation on HA from IAV strains with the known virulence and zoonotic potentials will help understand mechanisms of viral evolution and role of glycosylation in viral fitness. The fact that HA variants contain up to 12 N-glycosylation sequons means that the number of possible glycosylation variants is enormous. As a result, confident quantification of glycopeptides requires high quality tandem mass spectra of both intact glycoproteins and deglycosylated forms. Our workflow takes advantage of overlapping information from complementary analyses to generate high confidence results.

Keywords: Carbohydrates, Informatics, Liquid Chromatography/Mass Spectroscopy, Proteomics
Application Code: Biomedical
Methodology Code: Liquid Chromatography/Mass Spectrometry
### Abstract Text

Defining clinically relevant biomarkers for early-stage hepatocellular carcinoma (HCC) in a high-risk population of cirrhotic patients has potentially far-reaching implications for disease management and patient health. Changes in glycan levels have been associated with the onset of numerous diseases including cancer. In the present study, we used liquid chromatography coupled with electrospray ionization mass spectrometry (LC-ESI-MS) to analyze N-glycans in sera from 183 participants recruited in Egypt and the US, and identified candidate biomarkers that distinguish HCC cases from cirrhotic controls. N-glycans were released from serum proteins and permethylated prior to the LC-ESI-MS analysis. Through two complementary LC-ESI-MS quantitation approaches, global profiling and targeted quantitation, we identified 11 N-glycans with statistically significant differences between HCC cases and cirrhotic controls. These glycans can further be categorized into four structurally-related clusters, matching closely with the implications of important glycosyltransferases in cancer progression and metastasis. The results of this study illustrate the power of the integrative approach combining complementary LC-ESI-MS based quantitation approaches, to investigate changes in N-glycan levels between HCC cases and patients with liver cirrhosis.

### Keywords
- Biomedical
- Carbohydrates
- Liquid Chromatography/Mass Spectroscopy
- Proteomics

### Application Code
- Biomedical

### Methodology Code
- Liquid Chromatography/Mass Spectrometry
Protein glycosylation is an important and common posttranslational modification (PTM). More than 50% of human proteins are believed to be glycosylated to modulate the functionality of proteins. Aberrant glycosylation has been correlated to several diseases, such as inflammatory skin diseases, diabetes mellitus, cardiovascular disorders, rheumatoid arthritis, Alzheimer’s and prion diseases, and cancer. Many approved cancer biomarkers are glycoproteins that are not highly abundant proteins. Therefore, effective qualitative and quantitative assessment of glycoproteins entails enrichment methods. This presentation describes and discusses the use of different glycoprotein enrichment methods, including lectin affinity, hydrazide chemistry, and hydrophilic interaction chromatography. The use of these enrichment methods in assessing the qualitative and quantitative changes of glycoproteins in different diseases will be presented and discussed. The importance of glycoprotein enrichment techniques for the identification and characterization of new-reliable cancer biomarkers will be highlighted in this presentation. The use of these enrichment methods to assess the glycosylation of specific glycoproteins that play important biological functions, such as HIV gp 120, collagen and prostate specific antigen, will be described and discussed.

Keywords: Biological Samples, Capillary LC, Liquid Chromatography/Mass Spectroscopy, Tandem Mass Spec
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
The enumeration and characterization of circulating tumor cells (CTCs) in the peripheral blood may provide important prognostic information and might help to monitor efficacy of therapy: a real-time liquid biopsy. Since current assays cannot distinguish between apoptotic and viable CTCs, it is now possible to apply a novel ELISPOT assay (designated ‘EPISPOT’) that detects proteins secreted/released/shed from single epithelial cancer cells. Cells are cultured for a short time on a membrane coated with antibodies that capture the secreted/released/shed proteins that are subsequently detected by secondary antibodies labeled with fluorochromes. In prostate cancer patients (n=48), we used prostate-specific antigen (PSA) secretion as marker to detect PSA-secreting cells and observed that 83% and 42% of M1 & M0 cancer patients, respectively, had CTCs with a difference in the CTC median (29 for M1 & 9 for M0) and found that a significant fraction of CTCs also secreted fibroblast growth factor-2 (FGF-2), a known stem cell growth factor. More recently, in colon cancer, a considerable portion of viable CTCs detectable by the Epispot assay is trapped in the liver as the first filter organ in colon cancer patients. The enumeration of CK19-RC by the CK19-Epispot assay in 75 colorectal cancer patients revealed viable CTCs in 65.9% and 55.4% (p=0.04) patients in mesenteric and peripheral blood, respectively, whereas CellSearch detected CTCs in 55.9% and 29.0% (p=0.0046) patients. In mesenteric blood, the number of CTC was significantly higher than in the peripheral blood. Our clinical data showed that localized colon cancer patients with a high level of CTCs have an unfavorable outcome (n=60). In conclusion, the EPISPOT assay offers a new opportunity to detect and characterize viable CTCs in cancer patients and it can be extended to a multi-parameter analysis revealing a CTC protein fingerprint.
Isolation and Analysis of Circulating Tumor Cells (CTCs)

Multivalent Binding-Enabled Isolation of Tumor Cells in Microfluidic Devices

Circulating tumor cells (CTC) in the peripheral blood are potential biomarkers for cancer diagnosis and prognosis. However, CTC are extremely rare in the bloodstream, typically a few CTC in billions of normal blood cells, making their detection very challenging. To address this, we have developed a couple of multivalent binding schemes with microfluidic devices that are able to efficiently isolate cancer cells. One scheme is to create an optimized ensemble of aptamers and antibodies that function as a multivalent adhesive domain for the capture and isolation of cancer cells [1]. Due to their differences in size, aptamers and antibodies bind to different surface receptors on cells. As a result, their cooperative interactions with tumor cells result in higher cell capture efficiency than with either antibodies or aptamers alone. When incorporated into a microfluidic device, the ensemble showed not only high capture efficiency, but also superior capture selectivity at a high flow rate. The second multivalent binding scheme is to employ gold nanoparticles (AuNPs) as an efficient platform for assembling a number of aptamers, resulting in multivalent binding between a few aptamers with one tumor cell [2]. Up to 95 aptamers are attached onto each AuNP, and an increase of 39-fold in binding affinity with cells is obtained for AuNP-aptamer conjugates when compared with aptamers alone. In a flat channel microfluidic device, the capture efficiency of human acute leukemia cells from a cell mixture has increased from 49% using aptamer alone to 92% using AuNP-aptamer. In addition, we have employed microfluidic devices for detecting CTCs from peripheral blood samples of metastatic pancreatic cancer patients using CTC definition approved by FDA. We tested the potential utility of our devices for monitoring the patients’ response to anti-cancer drug treatment, and we found that the CTC numbers correlated with the clinical outcome.

Keywords: Bioanalytical, Biological Samples, Lab-on-a-Chip/Microfluidics, Nanotechnology
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
While metastatic disease causes ~90% of all cancer-related deaths, determining the role of various cancer cells, either from the primary tumor or those in circulation (circulating tumor cells, CTCs), in the metastatic process has been difficult due to the continuum of phenotypes they possess and/or the rare nature of these cells, especially CTCs. To assist in studies based on the use of rare CTCs that may be involved in metastasis, an integrated microfluidic system was designed, fabricated and evaluated. The system consisted of a CTC selection unit, an electrical detector for CTC impedance counting, an imaging unit for phenotyping single cells and a molecular analysis unit for detecting mutations or expression profiling certain genes of the CTCs. The CTC selection unit contained a series of 190 nL fluidic channels the walls of which were decorated with antibodies or aptamers used to recognize and select various sub-populations of CTCs from a single sample. The cell selection unit could process 7.5 mL of whole blood in ~20 min with a selection recovery of 97% and purity >80%. The CTCs could be released from the selection surface and initially counted by measuring impedance signatures of single cells that traversed through a pair of electrodes; CTC viability could be determined simply from the polarity of the impedance signature. The CTCs’ mRNA transcripts from certain genes, following impedance enumeration, were expression profiled using digital (single-molecule) counting that provided exquisite analytical sensitivity. The use of the system for determining the molecular characteristics of CTC sub-populations were evaluated for various cancers, including pancreatic cancer. For pancreatic adenocarcinoma, two sub-populations were analyzed; those with a more epithelial phenotype and those with an invasive phenotype with clear differences in their gene expression profiles.

Keywords: Bioanalytical, Biomedical, Lab-on-a-Chip/Microfluidics, Nucleic Acids
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Given these many therapeutic advances in medical oncology, the need to tailor treatment recommendations to each individual has never been greater. However, this personalization of cancer therapies requires biomarkers that 1) predict therapeutic benefit; 2) identify emerging mechanisms of resistance; and 3) tailor subsequent treatment strategies to continually evolving tumors. Successful development of predictive and pharmacodynamic biomarkers suitable for these purposes requires frequent sampling of tumor cells prior to, and throughout, the course of therapy. Circulating tumor cells (CTCs) are shed into peripheral circulation from primary and metastatic tumor sites and may represent a source of cells suitable for these purposes, without the need for invasive tumor biopsies. There is a need to develop technology with the capability to extract multiple analytes including protein, RNA and DNA, for the same molecular assays performed on tumor biopsies. Here we present, an exclusion based sample preparation CTC capture platform with integrated analysis known as VERSA (Vertical Exclusion-based Rare Sample Analysis) to allow further evaluation of proteins and nucleic acids. We demonstrate VERSA’s capabilities within prostate cancer focusing on the analysis of the androgen receptor (AR), which is a known driver of prostate cancer progression. Several AR targeting therapies have been developed and shown to be effective but there is now a critical need to evaluate and target mechanisms of resistance to these therapies. Using the VERSA platform we captured CTCs positive for the epithelial cell adhesion molecule (EpCAM) from castrate-resistance prostate cancer patients and quantified AR nuclear localization, as well as AR splice variant and AR dependent gene expression in CTCs as potential therapeutic biomarkers.

Keywords: Biomedical, Isolation/Purification, Lab-on-a-Chip/Microfluidics, Small Samples
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Isolation and Analysis of Circulating Tumor Cells (CTCs)

Selectin-Mediated Targeting of CTCs

Circulating tumor cells (CTC) have provided researchers with ample information about metastatic cancer. However, the exact mechanisms of metastasis are not definitively known. Mounting evidence indicates that CTC utilize the same pathway through which immune cells are recruited to sites of inflammation. This pathway involves a variety of adhesion molecules, including those in the selectin family. In this chapter, we discuss how these adhesion molecules, namely E-selectin, can be used to recapitulate the early steps of CTC adhesion in vitro to achieve capture and characterization of CTC from patient blood samples. We also discuss how this interaction may be exploited to target and kill CTC in vivo.

Abstract Text
Circulating tumor cells (CTC) have provided researchers with ample information about metastatic cancer. However, the exact mechanisms of metastasis are not definitively known. Mounting evidence indicates that CTC utilize the same pathway through which immune cells are recruited to sites of inflammation. This pathway involves a variety of adhesion molecules, including those in the selectin family. In this chapter, we discuss how these adhesion molecules, namely E-selectin, can be used to recapitulate the early steps of CTC adhesion in vitro to achieve capture and characterization of CTC from patient blood samples. We also discuss how this interaction may be exploited to target and kill CTC in vivo.

Keywords: Biomedical, Isolation/Purification, Lab-on-a-Chip/Microfluidics, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Microfluidics/Lab-on-a-Chip
Laser-induced breakdown spectroscopy (LIBS) has evolved as an important analytical technique for the detection and identification of elemental constituents by virtue of the atomic emission lines that are routinely captured in the visible region of the electromagnetic spectrum. However, certain environmental sensing applications demand higher specificity than can be achieved through the conventional LIBS approach. The transient thermal contrast that results by virtue of laser-deposited energy is known to produce a manifold of thermal infrared emissions containing the spectral fingerprint of the species that are present in the matrix. I hypothesized that similar molecular emissions would evolve when materials at the fringe of the LIBS plasma are thermally desorbed and heated by the plasma while retaining their integrity such that they emit their characteristic mid-infrared spectrum. In recent years we have undertaken empirical investigations of the thermal emission resulting from LIBS events in order to capture and characterize those molecular signatures that present concomitant to the LIBS phenomenon. The value of these recent laser-induced thermal emission (LITE) experiments is that they elucidated the time basis of the thermal emission under laser heating circumstances, revealing a critical time frame on the order of 100 ms during which the transient, non-equilibrium spectral signatures of molecular species are recoverable. I will discuss the origins of the hypothesis leading to these LITE experiments that have demonstrated its validity, and posit the exploitation of this phenomenology for environmental sensing applications.

Keywords: Environmental Analysis, Infrared and Raman, Spectroscopy, Surface Analysis
Application Code: Environmental
Methodology Code: Molecular Spectroscopy
Laser induced breakdown spectroscopy in the long wave infrared (LWIR) region can provide information on presence of specific chemical bond associated to certain material of interest, since many molecular vibration mode fall in to the LWIR region. Research activities have demonstrated the feasibility of acquiring LWIR spectra representative of the materials. However, the proof of concept setup requires long time and significant amount of sample to acquire the spectra. To explore further the potential of LWIR LIBS as an effective detection method, we designed and built LWIR LIBS instrument that integrates excitation laser and mercury cadmium telluride (MCT) linear array with readout IC. The usage of MCT array bounded to readout IC greatly reduced the size of the instrument and acquisition time, from tens of minutes down to a few seconds. The operation of the instrument is fully controlled by computer and microcontroller, whereas allowing user to specify all operation parameters. LWIR spectral of many materials were collected, such as KCl and energetic compound NH4CLO4. Acquisition of spectrum excited with single laser pulse was also demonstrated.

Keywords: Elemental Analysis, Molecular Spectroscopy, Near Infrared, Spectroscopy
Application Code: Homeland Security/Forensics
Methodology Code: Vibrational Spectroscopy
Long Wave Infrared Laser-Induced Breakdown Spectroscopy: LIBS beyond UV-VIS

MIR-LWIR Infrared (4-12 μm) Atomic and Molecular Emission Signatures from Inorganic and Organic Chemicals Using Laser-Induced Breakdown Spectroscopy

In an effort to augment the atomic emission spectra of conventional laser-induced breakdown spectroscopy (LIBS) and to provide an increase in selectivity, mid-wave to long-wave infrared (IR) LIBS studies were performed on several inorganic and organic chemicals. LIBS signature molecular emissions of target compounds are observed for the first time in the IR fingerprint spectral region between 4-12 μm. The IR emission spectra of select organic and inorganic chemicals closely correlate with their respective standard FTIR spectra. Intact and/or fragment sample molecular species evidently survive the LIBS event. The combination of atomic emission signatures derived from conventional UV-VIS-NIR LIBS with fingerprints of intact molecular entities determined from IR LIBS, promises to be a powerful tool for chemical detection.

**Keywords:** Laser, Pharmaceutical, Plasma Emission (ICP/MIP/DCP/etc.), Spectroscopy

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Molecular Spectroscopy
Emissions from the laser-induced breakdown spectroscopy (LIBS) plasma were examined in the region from 750 nm to 2000 nm. A Nd:YAG laser at 532 nm and 75 mJ per pulse was used to initiate the plasma. The detector was an InGaAs 1024 element diode array cooled to –100 C. A f/4 spectrometer with gratings blazed for this region was used as the dispersive element. Survey spectra of soils, uranium, and other selected samples were taken in air and in a flow cell purged with argon at the local pressure of 0.84 x 105 Pa. Strong infrared lines of neutral aluminum, carbon, potassium, silicon, sulfur and uranium, as well as once-ionized lines of calcium were observed out to 1670 nm. Time resolved measurements were performed with uranium samples in air and argon to study the temporal decay of the uranium lines and to search for molecular features. Detection limits of the IR system were compared with those obtained from a standard ICCD-spectrometer arrangement.

Keywords: Atomic Emission Spectroscopy, Laser, Nuclear Analytical Applications, Plasma Emission (ICP/MIP/DCP
Application Code: Homeland Security/Forensics
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Mid infrared time-resolved emission spectra were recorded from laser induced carbon plasma. These spectra constitute the first extension of carbon materials LIB spectroscopy into the mid infrared range. The carbon plasma was induced using a Q-switched Nd: YAG laser at pulse energy of 50 mJ at 1064 nm. Pulse width was 5 ns, repetition rate 10 Hz. The laser beam was focused to a spot size of 0.2 mm on graphite pellets mounted on a translation stage. Mid infrared emission from the plasma in atmospheric pressure helium gas was detected by a LN\textsubscript{2} cooled HgCdTe (MCT) detector in the range 8-11.6 \textmu m using a 7.4 \textmu m long-pass filter. The spectrometer was a 0.15 m focal length one with grating blazed at 8 \textmu m. LIB spectra were also taken in argon and also in nitrogen. Ten spectra were averaged using a boxcar. The resultant spectra were digitally smoothed. Even though gate delay of 10-20 microsecond and gate-width 16 microsecond were used there were strong backgrounds in the spectra. Part of the background may be due to radiation from hot carbon particulates. This background was empirically reduced using a baseline fit to the spectra. At a time delay of 2 microsecond no emission bands were seen. The thermal radiation due to the spectrometer housing was found negligible. Superimposed on this background broad bands were observed, the form and position of which were dependent on the ambient gas. In argon, for instance, bands were observed around 8.3, 9.5, 10 and 10.6 \textmu m. Carbon atomic lines CI may occur in this region, but these have small line strengths whereas a CII line with vacuum wavelength of 10457 nm could be seen in our spectra. Doubly ionized carbon has strong lines between 7900 and 9060 nm but high ionization is not expected under our experimental conditions. The width of the infrared bands is much larger than the spectral resolution of about 80 nm that indicates molecular origin. No linear carbon molecules (from 3 to 10 carbon atoms) have IR active vibrations in this range and the C\textsubscript{2} molecule cannot be detected as it is IR inactive. The infrared bands were thus compared to vibrational features of carbon clusters on the basis of quantum chemical calculations, previous carbon cluster infrared emission spectroscopic analyses in the literature and large carbon molecules observed in infrared astronomy. A plot of the carbon plasma in argon is shown here.

**Keywords:** Infrared and Raman, Plasma, Plasma Emission (ICP/MIP/DCP/etc.), Vibrational Spectroscopy

**Application Code:** Other

**Methodology Code:** Vibrational Spectroscopy
Microelectrodes, Microfluidics and Microdevices – Tools to Study Physiology On-Chip and In Vivo

Inter-Tissue Communication Using a 3D-Printed Device Reveals a Missing Link in Hormone Replacement Therapy in Diabetes

People with type 1 diabetes (T1D) must administer insulin exogenously due to the destruction of their pancreatic beta-cells. Endogenous insulin is stored as a crystalline hexamer in beta-cell granules along with C-peptide, a 31 amino acid peptide that is subsequently secreted from these granules in amounts equal to insulin. Exogenous co-administration of C-peptide with insulin has proven to reduce diabetes-associated complications in animals and humans, including complications related to microvascular blood flow and tissue health. Hypotheses for reduction in complications have included activation of eNOS and an increase in nitric oxide (NO) production in various tissues. However, the exact mechanism of C-peptide's beneficial effects after secretion from the beta-cell granules is not completely understood, thus hindering its development as an exogenously administered hormone. Here, we show that C-peptide alone does not affect bloodstream cells. Monitoring organ-to-tissue communication using a 3D-printed microfluidic device revealed that zinc and C-peptide are being delivered to erythrocytes by albumin. Upon delivery, erythrocyte-derived ATP increased by >50%, as did endothelium-derived NO, which was measured downstream in the 3D-printed device. Our results suggest that hormone replacement therapy in diabetes may be improved by exogenous administration of a C-peptide ensemble that includes zinc and albumin. We anticipate our results to be a starting point for more sophisticated studies involving C-peptide and insulin replacement therapy for people with T1D.

Keywords: Bioanalytical, Biological Samples, Biomedical, Biotechnology

Application Code: Biomedical

Methodology Code: Microfluidics/Lab-on-a-Chip
The regulation of oxygen levels in the brain is critical for normal function. Measurement of oxygen with microelectrodes provides insight into cerebral blood flow and metabolism, the two processes that are responsible for the supply and consumption of molecular oxygen. Our work has examined the regulation by neurotransmitters of cerebral blood flow, and the consumption of energy by neuronal activity. Additionally, we will demonstrate a microfabricated electrode that can be used to measure cerebral blood flow.

Keywords: Bioanalytical, Electrodes, Microelectrode, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
According to the US Centre for Disease Control in the US each year 1.7 million people suffer a traumatic brain injury (TBI) of whom 52,000 die, 275,000 are hospitalised and 1.3865 million are treated in the emergency room before release. TBI contributes to 31% of all injury related deaths in the US [1]. There is a pressing need to develop more effective clinical strategies for the emergency room and intensive therapy unit stages of clinical care. New analytical devices can be at the forefront of these new strategies [2].

We have developed a new class of clinical instrument that monitors in real-time electrical, physical and chemical information from the injured brain [3]. The system then searches for patterns of changes characteristic of the onset of secondary brain injury. Chemically we monitor changes in metabolism (glucose, lactate and pyruvate) and tissue excitability (potassium) using electrochemical biosensors and ion-selective electrodes respectively placed in a microfluidic manifold controlled by custom build electronics. The system is completed by an automatic calibration system built using a microfluidic circuit board (LabSmith Inc.) The microfluidic device analyses the dialysate stream from a clinical microdialysis probe (M Dialysis) placed near the site of brain injury using a cranial access bolt. The bolt also allow placement of a Clark electrode and temperature LiCOx probe (Integra) to give real-time measure brain oxygenation (pbt0[sub]2[/sub]).

By carefully comparing the relative timing of changes in these chemical signals, we can probe dynamic changes in brain metabolism in response to transient secondary insults to the injured brain (detected by the electrical and physical monitoring of the brain). We are optimistic that such an approach offers the prospects of individualised patient based therapies.


Keywords: Biosensors, Electrochemistry, Lab-on-a-Chip/Microfluidics, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Technical advances are making it possible to create tissue microenvironments on platforms that are compatible with high-content screening strategies. Recent collaborative work by our group has led to the development of microfabricated devices to enable culture of organized cellular structures which possess much of the complexity and function of intact intestinal tissue. Up to now, functional assays and screens for dietary factors, pathogens and drugs that affect the epithelia of the small and large intestines have been relegated to using traditional cell-culture methods and cancer-derived cell lines; however, these cells do not recapitulate many of the features of primary tissue, thus prohibiting physiologically relevant discovery. Breakthroughs in stem-cell biology now enable single stem cells or intestinal crypts isolated from primary mouse or human intestine to grow and persist indefinitely in defined 3D culture conditions to form organotypic structures generically termed enteroids. These 3D structures contain all of the expected lineages of the intestinal epithelium from which derived. Enteroid culture is conducted in large format culture dishes that render the cultures low-throughput, cost-prohibitive, and unquantifiable for screening. Our microengineered arrays and fluidic devices allow prolonged culture and experimental manipulation of these organotypic cultures. We have recently used organoids to generate millimeter-scale primary intestinal epithelium in vitro that more closely mimics the polarized 3D in vivo microarchitecture. These arrays can be interrogated by any of a variety of techniques including fluorescence, immunohistochemistry and genetic analyses. With the confluence of advances in bioanalytical technologies and protocols for long-term organotypic culture, these platforms are envisioned as next generation systems for high content assays of drug- and toxin-interactions with the intestinal epithelia.
One of the major thrusts in our research is exploring electrochemical measurements of cell metabolism and physiology. Clearly cellular dynamics and metabolism is a developing area where new instrumentation could have a significant scientific impact. In this regard, we created the multianalyte microphysiometer (MAMP) that employs electrochemical sensors to monitor near real-time metabolic rates of living cells in a microfluidic chamber with a concentration on cellular bioenergetics. In recent years, we have applied the MAMP to answer a wide variety of scientific questions with biomedical collaborators in neurology, immunology, and toxicology. At the same time, we are redesigning our previous MAMP instrumentation into completely Lab-on-a-Chip format entitled the “microclinical analyzer” (MCA). This MCA instrumentation is at the heart of the instrumented organs-on-a-chip multi-disciplinary effort of a DARPA, NIH UH2, and 2 DTRA grant awards that arise from the specific thrust of the current White House initiative to replace drug toxicology testing in animals with human tissues and organs. Coupling these microclinical analyzers with various organs on a chip will be the focus of this talk. Organs include the brain on a chip, the lung on a chip, the liver on a chip, and possibly others.

Keywords: Biosensors, Clinical/Toxicology, Microelectrode, Toxicology
Application Code: Clinical/Toxicology
Methodology Code: Electrochemistry
Coal tar is a viscous, complex mixture resulting from the pyrolysis of coal and has a wide range of uses from dermatologic products to fuel and asphalt sealants. When distilled, several creosote fractions can be collected and the remaining material is called “coal tar pitch”. It is commonly recognized that coal tar and creosote contain thousands of compounds. Yet studies on the environmental chemistry of these materials have been limited to the toxic polycyclic aromatic hydrocarbons (PAHs), the most abundant class present. Other compounds have been identified but generally limited to tentative identification with gas chromatography/mass spectrometry (GC-MS). To provide an expanded list of compounds in coal tar that would be invaluable to understanding the source, transport, and fate of these materials as well as constraining toxicological impacts, we analyzed field samples from coastal Texas using comprehensive two-dimensional gas chromatography (GCxGC) with flame ionization detection and time-of-flight mass spectrometry. The latter technique provides superior separation and quantification of GC-amenable molecules. By analyzing the whole extract of our field samples, we identified over 3000 peaks. (GC-MS only identified 250 peaks). We then fractionated the whole extract with silica gel chromatography into eight fractions. Analysis of each fraction led to the identification of over 10,000 peaks, although at trace levels, including numerous nitrogen-containing heterocyclic PAHs. The end result was a new and unprecedented inventory on the molecules present in coal tar samples that will lead to an increased understanding of a fossil-fuel product used almost exclusively in the environment.

Keywords: Capillary GC, Environmental Analysis, GC, Petroleum
Application Code: Environmental
Methodology Code: Gas Chromatography
Recent Advances in Molecular Characterization of Complex Industrial Matrices such as Oils and Polymers

Increasingly complex processes are used in refineries and petrochemical plants to transform heavier and unconventional oils into various products: ultrapure gases leading to technically advanced polymers, cleaner fuels, lubricants, bitumen... In order to optimize these (catalytic) processes, oils and hydrocarbon cuts have to be described at the molecular level. The origin of several polymer properties and efficiency of additives in polymers can be studied through their molecular characterization.[1,2]

The diversity of molecules found in oils and in polymers has stimulated the development of innovative chromatographic and mass spectrometric approaches to generate “fingerprints” at the molecular level. In this talk, we will present some of our latest developments in this field: Concerning heteroatom and metals containing molecules in crude oils, the complete distillation series of various crude oils have been studied using Gel Permeation Chromatography Inductively Coupled Plasma High Resolution Mass Spectrometry (GPC ICP HR MS).[3] Size distribution of sulfur, vanadium and nickel containing molecules or aggregates within an oil cut can then be determined, compared and studied through a refining process.

The coupling of atmospheric solid analysis probe (ASAP) with ion mobility-mass spectrometry (IM-MS) has been shown to be an efficient tool for the characterization of oil cuts,[4] polymers, polymer blends[1] and additives.[2] This approach affords the coupling of a direct ionization technique that does not require sample preparation, with a bi-dimensional separation method. It is therefore a useful tool for the rapid generation of molecular fingerprints from complex matrix samples.


Keywords: Fuels\Energy\Petrochemical, Hydrocarbons, Petrochemical, Polymers & Plastics
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Abstract Text

Characterization of aromatic compounds is very important in many research areas such as environmental, material, energy, and food researches. Especially, it is important to understand petroleum compounds. Mass spectrometry has been widely used for characterization petroleum compounds. Especially, high resolution mass spectrometry presented by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS) is a powerful technique to characterize compounds in complex mixture such as petroleum. The high resolving power and mass accuracy of FT-ICR MS enables accurate identification chemical compounds. In the high resolution mass spectra obtained from crude oils, over 10,000 peaks can be routinely identified. The elemental formulae of the compounds can be calculated and identified. However, to understand chemistry of the compounds, it is important to be able to assign structures to the identified elemental formulae. Therefore, in this study, the combination of HPLC, high resolution mass spectrometry and 2D NMR is proposed as a powerful technique to characterize petroleum compounds.

Keywords: HPLC, Mass Spectrometry, Petrochemical, Petroleum

Application Code: Fuels, Energy and Petrochemical

Methodology Code: Mass Spectrometry
Petroleomics is the characterization of petroleum at the molecular level. For sufficiently complete characterization of the organic composition of petroleum and its products, it should be possible to correlate (and ultimately predict) their properties and behavior. However, the comprehensive characterization of petroleum samples is still one of the most complex analytical challenges faced today and it mostly due to the lack of a universal analytical technique.

To overcome this limitation much progress has been accomplished by separating the matrix into distinct boiling point cuts, as described by the Boduszynski. He asserted that crude oil composition increases continuously with regard to aromaticity, molecular weight, and heteroatom content from the light distillates to non-distillables. The combination of distillation cuts with the molecular characterization provided by chromatographic and mass spectrometry analyses results in exhaustive characterization that provides compositional data that strongly supports the Boduszynski model. A more recent exhaustive characterization of ring number-separated fractions from a series of boiling point ranges has significantly expanded the molecular composition characterization of the aromatic components.

Although the molecular characterization provided by ultrahigh resolution mass spectrometry by direct infusion of crude oils may not provide a comprehensive characterization of species, it can provide insights and useful information if a comparative approach is undertaken. In this talk, we shall discuss how one can correlate and predict bulk properties of petroleum based on mass spectrometry analysis. We shall also describe our current efforts toward a new theoretical progression model that relies on direct infusion FT-ICR mass spectra of a whole crude oil and/or a distillation cut as an input, from which we are able to determine and extrapolate with high accuracy the molecular compositional space for a wide boiling point range.

Keywords: Characterization, Chemometrics, Fuels\Energy\Petrochemical, Mass Spectrometry
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Mass Spectrometry
Asphaltenes are a problematic component of crude oil that causes clogging of oil recovery pipelines and fouling of catalysts during crude oil processing. A better knowledge of their structures is imperative for addressing the problems they cause and also for finding potential uses for these compounds. Recent mass spectrometric studies of asphaltenes from around the world have shown that most ionized asphaltenes produce strikingly similar mass spectra upon collision-activated dissociation. However, previous studies of CAD of ionized model compounds have shown little resemblance to CAD of real ionized asphaltenes. We report here results obtained upon examination of new types of model compounds and different types of asphaltenes studied by using several different approaches.

Keywords: Ion Trap, Petroleum, Tandem Mass Spec
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Mass Spectrometry
**Abstract Text**

Sum frequency generation (SFG) vibrational spectroscopy has been applied to investigate buried interfaces involving polymers and proteins, supplemented by attenuated total reflectance (ATR)-FTIR. General methodology has been developed to probe buried polymer/metal interfaces, which are important for anti-corrosion coatings and packaging for modern microelectronics. SFG and ATR-FTIR have been used to examine molecular interactions between antimicrobial peptides and model cell membranes, and membrane associated proteins. Surface immobilized peptides and enzymes have also been studied at the solid/liquid interface in situ. Detailed correlations between the structure and function of these interfacial biological molecules have been obtained.

**Keywords:** Characterization, Protein, Surface Analysis, Vibrational Spectroscopy

**Application Code:** Bioanalytical

**Methodology Code:** Vibrational Spectroscopy
Scanning angle (SA) Raman spectroscopy is applied to study polymer films to simultaneously measure polymer thickness, chemical composition, structure and locate buried interfaces with tens of nanometer spatial resolution in the axial direction. The technique uses a prism on which the polymer is coated. Raman spectra are collected as the incident angle of the excitation laser is precisely varied from 30-70 degrees. This angular range spans above and below the critical angle for total internal reflection at a sapphire prism/polystyrene interface. SA Raman spectra of 10 nm to 2 micron polystyrene, polycarbonate or poly(3-hexylthiophene) films are collected with second acquisition times and signal-to-noise ratios that exceed 100. SA Raman spectroscopy is used to determine the interface location in bilayer films of polystyrene and polycarbonate. Comparing SA Raman and profilometry measurements, the average percent difference in the total bilayer thickness is 2.4% for films less than 2200 nm thick. The average percent difference in the thickness of the polystyrene layer, which reflects the interface location, is 2.5% when the polystyrene layer is at least 250 nm thick. SA Raman spectroscopy is a versatile method applicable whenever the chemical composition, structure and thickness of interfacial thin films needs to be measured with high axial resolution. This research is supported by the U.S. Department of Energy, Office of Basic Energy Sciences, Division of Chemical Sciences, Geosciences, and Biosciences through the Ames Laboratory. The Ames Laboratory is operated for the U.S. Department of Energy by Iowa State University under Contract No. DE-AC02-07CH11358.

Keywords: Raman
Application Code: Polymers and Plastics
Methodology Code: Surface Analysis/Imaging
Objective: Study the dynamics and structure of PTEN associated with laterally heterogeneous lipid bilayers using single molecule microscopy.

Background: Phosphatase and tensin homologue deleted on chromosome 10 (PTEN), is one of the most frequent genes deleted/mutated in a wide variety of tumors. PTEN acts as an antagonist to PI 3-kinase signaling, thereby affecting cellular processes such as cell proliferation and survival. PTEN activity is regulated by dynamic shuttling between the cytoplasm and the plasma membrane.

Methods: We use FTIR spectroscopy to monitor the secondary structure of PTEN and PTEN-L associated with complex lipid environments. In addition, we apply single-molecule TIRF microscopy and stopped-flow fluorescence measurements to observe PTEN molecules as they dynamically associate/dissociate and laterally diffuse along the lipid bilayer membrane. PTEN lipid binding is investigated using supported lipid bilayers of binary and ternary lipid mixtures of phosphatidylcholine (PC) with physiological relevant levels of phosphatidylserine (PS), phosphatidylinositol-4,5-bisphosphate (PI(4,5)P2) and/or phosphatidylinositol (PI).

Results: Using single molecule trajectories of PTEN molecules we find significant differences in PTEN dynamic behavior when bound to different membrane environments. Interestingly, we determine the existence of three membrane bound states with different diffusion coefficients. To gain insight into the molecular mechanisms of PTEN membrane association, we compared the lipid binding of wt-PTEN, PTEN-(1-15aa) and the recently discovered, PTEN-L with a 173 aa N-terminal extension. We find profound differences in the dynamic behavior of these PTEN derivatives at the membrane. In addition, we will present structural data of PTEN-L associated with plasma membrane lipids.

Keywords: Biospectroscopy, Fluorescence, FTIR, Lipids
Application Code: Biomedical
Methodology Code: Biospectroscopy
The controlled adsorption of proteins to defined monolayers has application in sensor and nanotechnology applications where selective adsorption and manipulation of the orientations of targeted species is of interest. Metal-chelating monolayers offer opportunities to manipulate the adsorption of metal-binding proteins and peptides, and in previous investigations of the binding of myoglobin to Cu(II)-charged 1,2-di(9-stearyryl)rac-glycero-3-triethyleneoxideiminodiacetic acid (DSIDA) monolayers via scattering methods, reorganization of the lipid layer was observed, although the molecular origins of this disruption could not be probed. In the studies reported here, vibrational spectroscopy methods were developed to shed molecular insights onto the origins of the monolayer disruption. Analysis of time-resolved planar array infrared reflection-absorption spectra (PA-IRRAS) revealed that a Cu(II) ion-chelated DSIDA lipid monolayer (Cu2+-DSIDA) was readily disrupted by adsorption of myoglobin as demonstrated by a blue shift of 1.7 cm⁻¹ and a lower intensity in the νas(CH₂) stretching mode of the lipid monolayer over a period of five hours.

In addition, we have used AFM-IR to investigate the interface between two and three concentric layers of a coaxially electrospun polymer nanofiber. The combination of atomic force microscopy (AFM) and infrared spectroscopy (IR) is a powerful tool that provides chemical and conformational information at a spatial resolution of 50-100 nm. Using an AFM-IR (Anasys) instrument we have studied cross sections of two and three layered fibers embedded in epoxy to assess the interaction between layers and the difference in crystalline content of the various layers. The potential of this technique for studying buried polymer interfaces will be discussed.

**Abstract Text**

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<th>Keywords:</th>
<th>Array Detectors, Polymers &amp; Plastics, Vibrational Spectroscopy</th>
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<td>Application Code:</td>
<td>Polymers and Plastics</td>
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<td>Vibrational Spectroscopy</td>
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Emulsions are very important in many industries including: oil refinery, cosmetics and food. The stability and properties of the emulsions are determined by the oil/water interface, which can be stabilized with surface-active species, such as surfactants or nanoparticles. The behavior of these species at the oil/water interface is known to effect emulsion formation and stability and can also be dependent on various other parameters such as concentration, ions, and additional cosurfactants and polymers. This presentation will describe our vibrational sum frequency scattering spectroscopy studies of surface-active species at the emulsion interface with a specific focus on the surfactant AOT. Its molecular structure, solvation and alkyl chain conformation as measured at the surface of a reverse emulsion particle suspended in an organic solvent, regular emulsion particle suspended in water and at the planar organic/water interface will be discussed as will the effect of counterion and particle size on the interfacial.
**Session Title**: CACA - How to be Successful in Your Career

**Abstract Title**: From a Research Scientist to a Group Manager, a President, and a Founder of Companies in the US and Taiwan

<table>
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<tr>
<th>Primary Author</th>
<th>Frank J. Yang</th>
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<td>Author</td>
<td>AcuTech Scientific Inc.</td>
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**Date**: Wednesday, March 11, 2015 - After Time: 01:35 PM

**Room**: 269

**Abstract Text**

A career goal is difficult to plan. Many excitement and accomplishment in one's career are difficult to plan, however, the success of one's career goal mostly comes from motivation and proactive action to become successful, hardwork, and support from your family, friends, and community networking. It is interesting to review my career path starting from $80 dollar in 1976 to arrive in Los Angeles Airport from Taiwan to study separation science with Professor Calvin J. Giddings at the University of Utah. With hard work and dedication to explore the fundamental of separation science, my path from a graduate student to establish my own company will be discussed. My time with Varain Associates, my venture into a small startup company, the merge of Lee Scientific with Dionex, the startup of FFFractionation company for Professor Giddings, the founding of my own MicroTech Scientific in Sunnyvale, California, the sales of MicroTech Scientific, and the Start up of AcuTech Scientific in US and Taiwan three years after my retirement had happen so fast. The excitement of the frontier research and innovation, the creation and the birth of new products, the mass production and expanded applications of the new products, the hard work and the networking to bring it to the real world keep the spirit high to achieve personal career goal of each Team members. My experience for getting funding for the start up in US and Taiwan will be discussed.

**Keywords**: Education

**Application Code**: General Interest

**Methodology Code**: Other
This presentation chronicles my personal career transitions after graduate school studying chromatography from post-doctoral fellowship in cancer research, chemical research, instrumental marketing to research in new in drug development. The continuing themes in my career are liquid chromatography, writing and teaching. I will describe my journey as a separation scientist in academic / corporate research, instrumental company, contract research organization and pharmaceutical industry as well as the impacts of two public organizations with profound influence on my development. The topics discussed will be scientific curiosity, career anchors, continuing learning, favorite authors, and megatrends / opportunities in chromatography and analytical chemistry.
Embracing the Uncertainty in Your Career

This is not a success story by any stretch of imagination. Rather, the presenter would like to share his journey--from a foreign student to a career in corporate America. Life always throws us curve balls, and we are constantly reminded to expect the unexpected. But if you so choose, you can find value and fulfillment in every circumstance--even the unexpected setbacks can bring new and positive possibilities. The key, though, lies in the constant preparation to embrace the change.

Keywords: Instrumentation
Application Code: Other
Methodology Code: Education/Teaching
Working in a global company can be challenging, especially for those originated from a different culture such as China. Based on personal experience, this presentation describes various challenges that a Chinese American R&D scientist might encounter and possible solutions to address these challenges.

1. Career goal
2. Innovation
3. Communication
4. Team work
5. Leadership
6. Networking

Abstract Text
Working in a global company can be challenging, especially for those originated from a different culture such as China. Based on personal experience, this presentation describes various challenges that a Chinese American R&D scientist might encounter and possible solutions to address these challenges.

Co-Author(s)

Keywords: Education
Application Code: General Interest
Methodology Code: Other
Ionophore-Based Chemical Sensors II

Quantum Dot Ion Sensors in a Printable Format

Water sources, including those in developed countries, continue to contain high levels of arsenic (As), at concentration levels exceeding the WHO drinking water guideline of 10 µg/L. Chronic exposure to arsenic through the consumption of these waters has been recognised to cause a world environmental health disaster, by putting 100 million people at risk of cancer and other arsenic-related diseases. This paper will report on the construction of a printed quantum dot sensor, using a paper-based or low cost optically transparent polymer coated substrate, with a dual quantum dot measurement system. A particle based ink will be discussed with an immobile carrier substrate for additional affinity ligands and a capture matrix for quantum dot sensors. A 3-D ‘porous-plug’ structure is produced to ensure complete and uniform interaction with the sample. The “ion-selective inks” have a carrier fluid which has been developed such that:

• It has viscosity suitable for fluidjet printing
• Forms uniform drops
• Does not dry at the print head nozzle but dries completely after printing.
• Enables particles to stay in suspension for sufficient time to allow printing.
• The total mass and volume occupied by the components of carrier solution for the ink are relatively low to allow for maximum loading capacity of the ink by the solid-phase ligand and sensor carrier.

QD nanoparticles are typically responsive to metal ions, mainly resulting in fluorescence quenching. The challenge here is therefore to achieve ion-selectivity for arsenic detection. CdTe core QDs and CdSe/ZnS core/shell QDs are compared and a dual QD system proposed to identify interfering ions such as Cu2+ and Zn2+. Taking inspiration from biology, peptide sequences have been sought to show affinity binding to As(III). Due to their high affinity towards complexation with As(III), glutathione (GSH) peptides were selected and are attached to the QDs, using carbodiimide chemistry. The ion-selective response is reported.

Keywords: Environmental Analysis, Fluorescence, Trace Analysis
Application Code: Environmental
Methodology Code: Sensors
Abstract Title: Measurement of Megakaryocyte-Released Serotonin by Ratiometric Enzyme-Based Phosphorescent Nanosensors

Abstract Text:
Imaging of thrombosis in animal models has advanced the understanding of pathophysiology, however gaps in knowledge still exist partly because of a deficiency of appropriate analytic tools. Existing methods almost exclusively use intravital microscopy with antibody-labeled coagulation proteins, cells, and cellular components. The limited quantitative potential of this approach necessitates the development of robust tools to visualize thrombosis at the platelet level by probing specific markers of platelet activation. Platelets possess receptors for serotonin on their surface and a serotonin transporter to re-uptake and secrete it during platelet activation, providing a suitable marker for monitoring platelet activation and thrombosis progression.

We have designed enzyme-based, ratiometric phosphorescent nanosensors specific to serotonin. The described non-ionic surfactant triblock copolymer-based nanosensors are comprised of a hydrophobic chain of poly(propylene oxide) flanked by a hydrophilic poly(ethylene glycol), and contains an O2-sensitive phosphorescent dye and reference. The surface of the nanosensors contains conjugated monoamine oxidase (MOA), which converts serotonin by an O2-mediated mechanism. We optimize the sensitivity, specificity over competing analytes, response time, and sensor lifetime of these nanosensors to yield tunable and reversible sensors of soluble serotonin via phosphorescence measurement of O2 consumption by MOA. We demonstrate that these nanosensors can quantitatively measure serotonin released from cultured platelet precursor megakaryocytes during IL-1-stimulated activation. These novel site-specific phosphorescent nanosensors can supply spatio-temporal information about serotonin release during thrombus development, providing a useful tool to advance our understanding of the pathophysiology of thrombotic complications.

Keywords: Biosensors, Imaging, Nanotechnology, Sensors
Application Code: Biomedical
Methodology Code: Sensors
Microfluidic paper-based analytical devices (µPADs) are becoming increasingly popular as simple to use and low-cost analytical tools for clinical or environmental applications. Our group has been focusing on drop-on-demand inkjet printing technology for both the patterning of microfluidic structures and the deposition of reagents required for analysis.

In most cases of µPADs realized up to date, highly porous materials composed of untreated cellulose fiber networks (e.g. filter paper, chromatography paper) have been applied, where the sample liquid is transported by wicking through the 3-dimensional network of fibers. While this concept has proven to be very successful, it has the disadvantage of being relatively slow. Depending on the configuration, the time required for the sample to completely wet a µPAD can take up to several minutes. Compared to fully enclosed conventional microfluidics, µPADs are commonly systems open to the environment, where liquid wicking competes with evaporation. As a consequence, comparably large sample volumes are required to guarantee the full wetting of the devices.

Here, we present an alternative approach to µPADs based on standard copy paper. In contrast to the devices mentioned above, microfluidic structures are patterned on the surface of hydrophobized copy paper. For this purpose, tetramethyl orthosilicate (TMOS) solution is inkjet printed to form hydrophilic flow channels. With this type of flow channel, significantly faster sample flow rates are achieved, due to a mechanism differing from simple capillary force-based wicking, allowing for shorter analysis times with smaller sample volumes. While in previously reported µPADs relying on filter or chromatography paper the sample is transported inside the paper network, the newly developed system relies on surface wetting. Finally, the use of copy paper instead of filter paper strongly contributes to the cost-reduction of µPADs, since the paper substrate is a significant cost factor.

**Keywords:** Bioanalytical, Biological Samples, Chemical, Lab-on-a-Chip/Microfluidics
**Application Code:** Bioanalytical
**Methodology Code:** Microfluidics/Lab-on-a-Chip
After a long history and conflicting views, solid contact (SC) liquid membrane ion-selective electrodes (ISEs) emerged as reliable potentiometric sensing devices with unique advantages. The main motivation for the development of SC electrodes is miniaturization through microfabrication. Solid contact microelectrodes are expected to be robust, maintenance free or single use that does not need calibration.

In the light of these expectations it is surprising that most papers describe the properties of the SC electrodes only after extensive, sometimes complex, equilibration/conditioning processes. However, in certain applications (single use sensors), short equilibration time is essential. In other applications (in vivo measurements requiring sterilized sensors), complex and long conditioning may not be feasible. In addition, understanding the limits of the beneficial effects of conditioning is important (experimental conditions, time). Finally, if the electrode properties change during use and storage (wet or dry) it is important to know how can the original properties restored (reconditioning).

We studied the equilibration processes of freshly prepared potassium, sodium and hydrogen ion-selective electrodes with inherently conductive polymers (CPs) as inner contacts. As conductive polymers poly (3,4-ethylenedioxythiophene), poly(3-octylthiophene), and polypyrrole were used. The CPs were deposited over gold, platinum and glassy carbon substrates by drop casting, electropolymerization or by cyclic voltammetry. The electrodes were characterized by their equilibration time, (the time instant when the potential drift drops below a threshold value), short- and long-term stability. Finally we evaluated the reproducibility of the equilibration process following the complete exsiccation of the solid contact electrodes.
Label-free detection of polyions such as sulfated polysaccharides (e.g., heparin) and arginine-rich proteins (e.g., protamine) is challenging owing to the lack of fluorophores, chromophores, or redox active groups in these polymeric species. Polymer membrane electrodes doped with appropriate ion-exchangers or lipophilic salts have proven to be useful sensors for detecting such polyions. However, chromatographic separation is required when different polyions co-exist, or the polyion sample contains significant levels of interfering ions. In this presentation it will be demonstrated that a fully reversible polyion sensor can be used as a simple potentiometric detector to monitor separated polyions eluted from a chromatographic column. A polymer membrane doped with tridodecylmethylammonium-dinonylnaphthalene sulfonate (TDMA/DNNS) is employed as the membrane electrode detector and is operated in a triple-pulse mode. This allows for the fully reversible detection of either polycations or polyanions eluting in the eluate from the column, depending on the direction of the initial current pulse used in the detection pulse sequence. In proof-of-principle experiments, homogeneous poly-arginines of different lengths are separated by using a heparin-sepharose affinity chromatography with a NaCl gradient, and these poly-arginines are detected as positive EMF bands as they elute from the column. The same system can also be applied to monitor the separation of arginine-rich polypeptides produced by thermolysin-catalyzed digestion of protamine. Based on the strong electrostatic interaction of polycations and polyanions, it will further be shown that the polyion-sensitive pulstrode can also be employed for the indirect detection of polyions separated on chromatographic columns using a post-column polyion reagent approach.


Keywords: Chromatography, Electrochemistry, Electrodes
Application Code: Biomedical
Methodology Code: Electrochemistry
Heparin is widely used as an anticoagulant during procedures such as cardiac/vascular surgery and kidney dialysis. Adverse reactions, including many deaths, resulted when tainted heparin was accidentally used for the treatment of patients in 2007-08. The contaminant that was responsible for the adverse reactions was later identified to be a high charge density polyanion polysaccharide called oversulfated chondroitin sulfate (OSCS)[1]. After this incidence, testing of solutions of heparin for purity before using for patient treatment has been a requirement. However, since OSCS has similar chemical structure and anticoagulant activity as heparin, it cannot be detected in heparin solutions by typical bioactivity assays. Thus, currently, only costly and complex techniques, proton nuclear magnetic resonance (NMR) spectroscopy and capillary electrophoresis (CE) are mandated by FDA to screen for such contaminants in heparin. Simple and more easily available potentiometric polion sensors were recently reported for the detection of OSCS in heparin.[2] However, these are single use disposable devices and direct quantitation of the contamination level is difficult. We present here pulsed chronopotentiometry with polion-selective electrodes as a simple, reversible and reliable method of detection of high charge density contaminants in heparin solutions. In our preliminary work, as low as 1% dextran sulfate, a high charge density polysaccharide (similar with OSCS in structure), has been detected in heparin solution at 2.3 mg/ml.

References:

Keywords: Biomedical, Electrochemistry, Ion Selective Electrodes, Sensors
Application Code: Biomedical
Methodology Code: Electrochemistry
A New Platform for Nanomolar Detection of the Neurotransmitter Acetylcholine Potentiometrically via the Inclusion of Water-Soluble p-Sulfonatocalixarene in the Electrode Inner Filling Solution

Developing ion selective electrodes (ISEs) with a nanomolar detection limit for online monitoring of the neurotransmitter acetylcholine (ACh) in biological fluid is presented here. Our strategy depends on optimizing the ISE inner filling solution by buffering the activity of ACh and consequently reducing the transmembrane ion flux. This has been achieved through utilizing the outstanding complexation properties of water-soluble p-sulfonatocalixarenes towards the quaternary ammonium ACh cation to form host-guest inclusion complex. UV-spectroscopy was used to investigate the formation of the proposed complex and its leaching to the outer sample solution was tracked. The performance characteristics of the developed ISE revealed a linear range from 1 mM to 1 nM with LOD of 7.6×10⁻¹⁰ M which is lower than the ACh basal physiological level. Moreover, the developed ISE showed good temporal resolution and selectivity for ACh in the presence of the anticipated biological ions. To investigate the ability of the ISE to detect ACh in real samples, ACh has been determined in artificial cerebrospinal fluid (aCSF) samples spiked with ACh at a concentration comparable to its physiological level.

Keywords: Analysis, Bioanalytical, Electrodes, Potentiometry
Application Code: Bioanalytical
Methodology Code: Sensors
Super-resolution chemical imaging via Raman spectroscopy offers chemical specificity, affording the ability to multiplex numerous label-free analytes while providing a spatial chemical distribution on sample. However, spontaneous Raman is inherently weak making trace detection and thus super-resolution imaging extremely difficult, if not impossible. To circumvent this and allow for a trace detection of the few species present in each sub-diffraction limited location, we have developed a coherent fiber-optic imaging bundle based surface enhanced Raman scattering (SERS) probe consisting of 30,000 individual fiber elements. When tapered, etched and coated with metal, they allow circular Raman chemical images with 20µm diameters to be obtained from the array of 50 nm individual fiber elements. An acousto-optic tunable filter is used to rapidly scan or select frequencies of interest for multi or hyperspectral analysis.

Although the 50nm fiber element dimensions of this probe are capable of inherently providing spatial resolutions of approximately 100nm, further increases in the spatial resolution can be achieved by using a rapid, 2-step dithering process. Using this process, two additional images are obtained one-half fiber diameter apart in the x- and y- plane. A piezostage drives the movement of the stage and allows accurate and reproducible shifting of the images. These images are then deconvoluted from each other to further increase the spatial resolution to one-third of its original value. This talk will describe super-resolution chemical imaging using these probes and the dithering method as well as its applications in label-free imaging lipid rafts and other applications within biology.

Keywords: Acoustic-Optic Tunable Filter, Fiber Optics, Raman, Surface Enhanced Raman
Application Code: Bioanalytical
Methodology Code: Surface Analysis/Imaging
Raman imaging is fast becoming one of the key pillar techniques used in material characterization due to its sensitivity, speed, spatial resolution and highly detailed chemical and structural information content. The advantages of Raman imaging of advanced materials are illustrate particularly well for the analysis carbon based nanomaterials (carbon nanotubes, graphene and graphene oxide), thin films and coatings. Raman imaging provides a wealth of information beyond just simple identification and includes detailed structural information. Raman imaging can be used to distinguish between different states of materials such as crystalline, amorphous, and nano-structured materials as well as observing subtle effects such as changes induced by applied stress such as those observed in thin layers of silicon deposited on germanium doped silicon substrates. This presentation will highlight Raman imaging results on several of these materials with a detailed discussion on the information that is contained in the resultant images.
The physical and biological properties of organic solids can be profoundly affected by crystal form. Experimental measurements by second harmonic generation (SHG) microscopy suggest rapid transitioning through metastable crystal forms at the earliest stages of crystallization before ultimately producing the most thermodynamically stable crystal form. This trend supports a >100 year old theory by Wilhelm Ostwald that has historically been challenging to test. Advances in measurement capability are described from coupling SHG microscopy directly into a beamline for synchrotron X-ray diffraction. The regions of interest identified by SHG microscopy were targeted for X-ray diffraction, enabling structural analysis of pg quantities of crystalline material. By confining crystallization to small volumes, inkjet printing places crystallization under kinetic control. As such, metastable crystals forms that would otherwise transition to stable forms could be kinetically trapped to provide snapshots of structures present during the early stages of crystallization. When inkjet printing racemic amino acid solutions, the XRD measurements confirmed spontaneous resolution of the crystal forms into separate homochiral crystals when crystallization was performed under kinetic control through inkjet printing. Metastable crystal formation has also been observed in nominally centrosymmetric materials, including crystals of a centrosymmetric dye molecule. These results may allow for new, efficient routes for chiral resolution in chemical syntheses.

Keywords: Amino Acids, Instrumentation, Pharmaceutical, X-ray Diffraction
Application Code: Pharmaceutical
Methodology Code: Surface Analysis/Imaging
Currently, the most commonly used method for characterizing binding materials in paint cross sections is GC-MS; however, the analysis requires an extensive extraction and derivatization process and is destructive to the sample. Recently, research has been focused on developing less-destructive methods to determine paint binding media. ToF-SIMS has proven to be one effective method for the simultaneous identification of both binding materials and pigments in cross section. However, ToF-SIMS requires a high vacuum and a high energy ion beam in order to produce secondary ions for imaging. Desorption electrospray ionization [DESI], on the other hand, can be operated in atmospheric conditions, as ionization is induced by a charged solvent spray using a front end source that can be fitted to most mass spectrometers. In this talk, preliminary results for the DESI-MS identification of binding materials such as oil, egg tempera, and acrylic media will be discussed. DESI-MS can be used to create isobaric images to visualize the location of different binding material in a prepared cross-section with a spatial resolution of approximately 100 µm. This DESI-MS method has also been successfully used to analyze a cross section from a 17th century baroque painting currently undergoing restoration at Villanova.

**Abstract Text**

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**Keywords:** Art/Archaeology, Identification, Imaging, Mass Spectrometry

**Application Code:** Art/Archaeology

**Methodology Code:** Mass Spectrometry
This presentation will give an overview of the non-destructive, qualitative elemental analysis of high-Z particles embedded in a mineralogical sample using two laboratory-based X-ray techniques: micro X-ray computed tomography (micro-CT) and confocal micro X-ray fluorescence spectroscopy (confocal micro-XRF). Traditional micro-XRF instruments are limited in that they cannot distinguish surface from sub-surface particles. The limitations of micro-XRF can be overcome by confocal micro-XRF, which allows for spatial discrimination of X-ray fluorescence signals in all three spatial axes as well as particle detection and elemental identification at depth. Data is acquired by rastering the sample; hence, large samples can require days to fully acquire a 3D elemental image. This analysis time can be significantly reduced by screening the samples using micro-CT, a non-destructive X-ray imaging technique. This technique rapidly collects absorption contrast images of a sample and reasonable 3D data sets can be acquired within a few hours. While discrete elemental information is difficult with micro-CT, particles composed of high-Z elements can be differentiated from particles composed of low atomic number elements based on X-ray attenuation. Using the particle location information, the prescreened particles can be targeted individually for in situ elemental analysis which rapidly decreases the time required to locate and elementally identify particles of interest. In this case study, a cylindrical mineral sample was imaged non-destructively using micro-CT. Two high-Z particles (~20 μm in diameter) were located (one surface particle and one sub-surface particle) and elementally identified using confocal micro-XRF. These results show that coupling these two X-ray based imaging techniques allows for the spatial location, size measurement and elemental identification of high-Z particles within a matrix, in a non-destructive manner.

Keywords: Elemental Analysis, Imaging, Microscopy, X-ray Fluorescence
Application Code: General Interest
Methodology Code: X-ray Techniques
Advances in Surface and Imaging Analyses

Expanding the Surface and In-Depth Capabilities and Applications of X-ray Photoelectron Spectroscopy (XPS) with a Monatomic and Gas Cluster Ion Source (MAGCIS)

X-ray photoelectron spectroscopy (XPS) is a powerful surface analytical technique that provides unique chemical state information on the topmost ~2-10 nm of solid materials. In-depth compositional information can be obtained up to depths of several micrometers when XPS is combined with argon ion beam sputtering for depth profiling analyses. However, ion beam sputtering of most organic and polymeric materials, and certain inorganic materials, can often be problematic when using monatomic argon ion beam sources because of chemical damage caused by the ion beam sputtering process. This damage greatly reduces the chemical information to be learned from XPS depth profiling.

Recently developed argon cluster ion beam sources offer exceptionally gentle sputtering compared to monatomic sources and produce much less chemical damage when used to depth profile or sputter-clean ion beam-sensitive materials. Hence, cluster ion sources allow materials that are unstable under monatomic ion bombardment to be successfully depth profiled. This presentation will discuss applications of a unique combined monatomic and gas cluster ion source (MAGCIS) that can be operated as either a traditional monatomic argon ion source or as an argon cluster ion source, thereby allowing a full range of material types to be successfully analyzed. XPS depth profiling results from a variety of biological, organic, and inorganic materials will be described with comparisons to results obtained for samples analyzed using monatomic argon ion sputtering. Results obtained with argon cluster ions were consistent with minimal ion beam damage and preservation of important chemical state information in the resulting XPS spectra.

Keywords: Materials Characterization, Nanotechnology, Polymers & Plastics, Surface Analysis
Application Code: Nanotechnology
Methodology Code: Surface Analysis/Imaging
Bioanalytical - Vibrational Spectroscopic Applications

**Abstract Title**: Analysis of Drugs in the Saliva During Treatment of Military Veterans Suffering from Post-Traumatic Stress Disorder

**Primary Author**: Chetan Shende  
Real-Time Analyzers, Inc.

**Co-Author(s)**: Stuart Farquharson

**Keywords**: Biomedical, Clinical Chemistry, Portable Instruments, Surface Enhanced Raman Spectroscopy

**Methodology Code**: Vibrational Spectroscopy

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Over the past decade, the inundation of the US with illicit drugs and the continued erosion of attitudes regarding the harm of such drugs have resulted in an increase in their abuse along with prescription drugs. Military veterans represent a special case, in that some 476,000 have received treatment for post-traumatic stress disorder and developed drug dependence. Treatments for drug dependent patients include drug dose lowering, medications to remove drug dependence and/or alleviate drug-related symptoms. Veteran Affairs hospital physicians must frequently test patients to identify discontinuation of medications or any recurrence of drug use, and adjust treatment appropriately. These tests most often involve collecting and sending a urine sample to a clinical lab for analysis by gas chromatography coupled mass spectrometers. Results are usually returned in 1 week, a delay that makes timely adjustment of treatment difficult. In an effort to develop an analyzer that can determine drug use at the time of VA hospital or clinic visits so that treatment can be adjusted, we have been developing a surface-enhanced Raman spectroscopy (SERS) based assay that can detect and identify numerous drugs in saliva at ng/mL concentrations within 10 minutes. Measurements of representative illicit, prescribed, and over-the-counter drugs in saliva by SERS, with a focus on cocaine, will be presented.
Surface-enhanced Raman scattering (SERS) is a promising analytical technique for the detection and characterization of biological molecules and structures. In this presentation, approaches for label-free protein detection using SERS will be discussed. The sample preparation step is the key point to obtain strong and reproducible SERS spectra from the biological molecules and structures. When the colloidal suspension is used as a SERS substrate for the protein detection, the electrostatic interaction of the proteins with the nanoparticles is described by the nature of their charge status, which influences the aggregation properties such as the size and shape of the aggregates, as well as orientation of nanoparticles in the aggregates, which is critical for the SERS experiment. However, when the solid SERS substrates are fabricated, SERS signal of the proteins that are background free and independent of the protein charge. Pros and cons of using colloids and surfaces as SERS substrate will be discussed for label-free detection of proteins using SERS.

Keywords: Bioanalytical, Proteomics, Surface Enhanced Raman, Vibrational Spectroscopy

Application Code: Bioanalytical

Methodology Code: Vibrational Spectroscopy
**Abstract Text**

The May 2013 mailing of Ricin to President Barack Obama, New York City Mayor Michael Bloomberg and, Director of Mayors against Illegal Guns Mike Glaze once again demonstrated the need for a bioagent analyzer to protect US citizens at home and the military abroad. Despite the substantial effort to develop bioagent analyzers, current analyzers 1) are too slow (e.g. PCR, 1 measurement/hour), 2) have high false-alarm rates (e.g. immunoassays), 3) lack sensitivity (e.g. Raman), 4) are not field-usable (e.g. GC-MS), or 5) cannot be multiplexed to identify multiple species (e.g. PCR). In an effort to overcome all of these limitations, we have developed a sample system that selectively binds specific bioagents and produces surface-enhanced Raman spectra. Personnel at the US Army’s Edgewood Chemical Biological Center have used this sample system coupled with a compact, portable Raman spectrometer to measure 100 B. anthracis Ames spores in 20 minutes. These measurements, along with measurements of other Bacilli that demonstrate discriminate analysis, will be presented.

**Keywords:** Bioanalytical, Biological Samples, Portable Instruments, Surface Enhanced Raman

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Vibrational Spectroscopy
Both surface plasmon resonance (SPR) and total internal reflection ellipsometry (TIRE) has been successfully applied for the evaluation of protein-protein interaction. TIRE combines the assets of both spectroscopic ellipsometry and surface plasmon resonance (SPR). Therefore, it has a significant advantage due to simultaneous measurements of two ellipsometric parameter which are related to the amplitude and phase shift of p- and s-components of polarized light respectively [1,2]. In here presented research total internal reflection ellipsometry (TIRE) and SPR has been applied for the evaluation of: (i) kinetics of biosensing layer formation, which was based on the immobilization of fragmented and intact antibodies, and (ii) kinetics of antigen interaction with the immobilized antibodies. The ellipsometric parameter showed much higher sensitivity at the initial phase of Au-protein and protein-protein interaction, while the parameter was more sensitive when the steady-state conditions were established. The optical response of ellipsometric parameters in TIRE method has shown that the sensitivities of and were different at different stages of biomolecular layer formation.

Acknowledgement: Supported by Research Council of Lithuania, Support to research of scientists and other researchers (Global Grant), Enzymes functionalized by polymers and biorecognition unit for selective treatment of target cells (NanoZim’s), Project Nr. VP1-3.1-ŠMM-07-K-02-042.


Keywords: Biosensors, Biotechnology, Sensors, Surface Analysis
Application Code: Bioanalytical
Methodology Code: Surface Analysis/Imaging
In the biopharmaceutical industry, it is needed to make high concentration (>100 mg/ml) protein formulations. So, to understand the characteristics and their changes of protein as solute in non-ideal solution may contribute to give important suggestions for improvement of stability of biomedicine solutions.

In this study, Raman spectroscopic measurements of lysozyme solution as model system over a wide range of concentrations (2.5 mg/ml – 300 mg/ml) were carried out to investigate the molecular structure and the interaction of protein in highly concentrated solution. In this wide range of concentration, the specific Raman bands (Amide I, Tyr and Trp) were focused on and analyzed. The experimental results revealed that the molecular interaction changes in the stepwise processes as an order of magnitude of the distance between molecules changes. These results suggest that the practically effective maker bands are capable of evaluating appropriate formulation of the highly concentrated solution of protein, and also suggest the potential of Raman spectroscopy which will bring progress not only in molecular science in the concentrated, e.g. crowded condition but also in the protein process industry, such as biopharmaceutical field.
The thermal denaturation mechanism and secondary structures of two kinds of human insulin nanoparticles, which are produced by supercritical fluids with ethanol or DMSO solutions, are investigated by applying principle component analysis (PCA), two-dimensional (2D) correlation spectroscopy and all-atom molecular dynamics (AAMD) simulation. 2D correlation spectroscopy combined with PCA enables improved characterization of the structural changes in biomolecules on a detailed molecular level. AAMD simulation method is a powerful tool that enables the examination of complex systems and processes at the atomistic level. The results of the PCA, the 2D correlation spectroscopic analysis, and the AAMD calculations clearly revealed that the thermal denaturation mechanisms and the degrees of hydrogen-bonding in the spherical and rod-shaped insulin nanoparticles were different. The details of differences of two kinds of human insulin nanoparticles will be discussed in this presentation.

Keywords: Analysis, Characterization, Computers, FTIR
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Synchrotron infrared nano-spectroscopy (SINS) enables spectrochemical imaging over the full mid-infrared range with sub-40 nm spatial resolution (Bechtel et al. PNAS 2014, 111:7191-7196). With SINS, we have now extended our micro-scale assessment of fungal wall composition with nanoscale comparisons of wall and exudate composition. Fungi are ubiquitous micro-organisms that collectively have profound effects, both positive and negative, on the environment and on human biology. Fungal cells are protected by walls that enable penetration of their surroundings, plus exudates that include adhesins and hydrolases. Despite concerted efforts to resolve and identify the chemical constituents of cell walls and fungal exudates, much remains completely unknown. One of the key challenges is that the hyphal wall composition changes along the length of the hypha as the cell matures. Fungal wall sugars are not found in animal cells, so wall biosynthesis pathways are good anti-fungal drug targets. In this study, we are examining walls and exudate in wild type, ugmA knockout, and alpha-glucan synthase knockout Aspergillus strains. We have preliminary evidence that (1) exudate contains both carbohydrate (adhesion) and protein (nutrient acquisition) and (2) carbohydrate composition is radically changed in strains lacking genes responsible for biosynthesis of critical minor compounds; results can be correlated with molecular cell biological composition studies. We report here results of the first nanoscale FTIR imaging study (well beyond the diffraction limit), together with AFM morphological analysis, of fungal cell walls and exudate.
### Optimizing an Infrared Spectroscopic Method for QC Analysis of Botanical Dietary Supplements

Botanical dietary supplement manufacturers are now subject to the FDA regulatory guidelines for good manufacturing practices. The cGMP’s require verification of all incoming raw materials, and compliance with 21 CFR Part 11 for electronic record and signatures. The pharmaceutical industry relies upon validated Pharmacopeia methods for testing, however; this is not the case for dietary supplement manufacturers. One accepted way of identifying raw materials is to analyze the sample by infrared spectroscopy. A spectrum of standard material is collected and compared statistically to a spectrum of the unknown sample for quality of fit.

The raw materials may be sourced from various locations around the world and their characteristics may fluctuate due to harvest, weather, and soil conditions from country to country and year to year. These sources of variability will be observed in the infrared spectrum of the material and will decrease the probability of correctly identifying the material. A variety of different infrared sampling techniques were applied to the samples in order to determine the optimum method for performing this analysis. The spectra obtained from the botanical dietary ingredients were challenged to test the suitability of infrared spectroscopy to recognize and verify an individual dietary ingredient.

Chemical extraction of the material from the sample matrix can be performed prior to infrared analysis, but is a lengthy process. The paper proposes a rapid method of accomplishing the same result by digitally extracting the spectrum of the raw botanical material from the bulk material, thereby reducing the problems of product variability and greatly enhancing the ability to positively identify the material.

### Keywords
- Analysis, FTIR, Pharmaceutical
- Consumer Products
- Vibrational Spectroscopy
In liquid phase microextraction, high enrichment factors (EFs) can be obtained using an acceptor phase of small volume. By hanging an acceptor drop at the separation capillary tip, single drop microextraction (SDME) can be in-line coupled with capillary electrophoresis (CE). The small surface-to-volume ratio of the drop enables high EFs to be obtained in a short time. One practical issue in SDME is how to keep the drop attached to the capillary stably. Here, we present novel but extremely simple in-tube microextraction (ITME) using the liquid inside the capillary as an acceptor phase, without forming a drop at the capillary tip. In this report, we demonstrate headspace (HS) extraction-ITME-CE fully automated using a commercial CE instrument. As a first example of HS-ITME-CE, chlorophenols in an aqueous sample solution were extracted into the acceptor plug in a separation capillary, simply by placing the capillary filled with a basic run buffer in the HS above the sample, and then electrophoresis of the extracts in the capillary was carried out. Owing to the robust nature of the acceptor phase, the extraction temperature and time ranges of HS-ITME can be extended significantly, compared to HS-SDME. The EFs for chlorophenols in a standard solution were up to 1100 under an optimal HS-ITME condition of 80°C for 15 min and the limits of detections (LODs) obtained using a built-in UV detector were about 4 nM. HS-ITME-CE was also applied to extract neutral organic pollutants such as BTEX (benzene, toluene, ethyl benzene, and xylene) from an aqueous sample using an acceptor plug of a surfactant solution. The extracts were then analyzed with a micellar electrokinetic chromatography mode of CE. The EFs for BTEX obtained with 20 min HS-ITME at room temperature were about 340 and the LODs were about 3 ppb.

Keywords: Capillary Electrophoresis, Extraction, Headspace, Volatile Organic Compounds
Application Code: Environmental
Methodology Code: Capillary Electrophoresis
Phospholipid bilayers have been employed very successfully for some time in capillary electrophoretic (CE) separations. They have been shown to be highly stable, semi-permanent, coatings and are ideally suited to prevent the adsorption of proteins and other molecules to the capillary surface. The phosphocholine lipids also offer a unique level of electroosmotic flow (EOF) control. These zwitterionic surfactants, such as 1,2-Dilauroyl-sn-glycero-3-phosphocholine (DLPC), are able to yield a significantly suppressed EOF under typical separation buffer conditions, but when calcium is added to the separation buffer, the EOF is reversed.

The need for calcium to be present in solution with the phospholipids has long been known, and calcium is crucial for the formation of stable phospholipid bilayers. The influence of excess calcium on the EOF, resulting in not only a reversal, but also an increase in the magnitude of the EOF. This indicates that the presence of the calcium ion in the separation buffer leads to a partitioning of some of the calcium into the phospholipid bilayer, increasing the cationic charge of the bilayer and altering the EOF. This leads to the question of how other metal cations might influence the magnitude and direction of the EOF when present in the separation buffer.

To answer this question we have investigated the influence of a range of metal chloride salts on the EOF that is achieved when they are included in the separation buffer in the place of calcium. As anticipated, the charge of the metal cation plays a significant role in the magnitude of the EOF, with trivalent cations such as cerium drastically increasing the EOF. Unexpectedly, not all divalent cations yield an equivalent magnitude for the EOF. This presentation will detail our results of these investigations as well as our study of the stability of the bilayers with these different cations, and their application to the separation of various anionic and cationic proteins.

Keywords: Bioanalytical, Capillary Electrophoresis, Characterization, Membrane
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
CE and Microfluidics

Woven Fabric as a Low-Cost Microfluidic Platform for Tuned Electrophoretic Separations

Low-cost microfluidic devices have been widely researched as potential ways to making medical diagnostics and therapeutic monitoring more accessible to low income and low resource areas of the world. Current approaches to low cost device manufacture involve the assembly of materials such as chromatographic paper into microfluidic devices in multiple manufacturing steps or using microfabrication techniques such as UV-photolithography, which are expensive and difficult to scale up. In prior work, we demonstrated a textile weaving-based approach that makes use of this scalable and readily accessible technology to manufacture microfluidic devices in a single step. Liquid flow was tuned in these devices by manipulating the wetting characteristics and chemical treatment imparted to yarns in a seamless manner along the length of the fabric. In recent work, we leveraged this tuning ability to manufacture fabric-based electrophoretic devices for the separation of mixtures of proteins. Electrophoresis is widely used for the pre-concentration, separation and assay of protein analytes in complex samples. We present the ability to tune separation resolution using the surface properties (wetting characteristics) and the packing density (number of yarns per unit area) of both woven and knit fabrics. A seamless transition in yarn surface properties and packing density may then be used to perform protein sample pre-concentration followed by separation in a single fabric device, with potential applications in the detection of low abundance (rare) protein markers from clinical samples at the point-of-care or in low resource settings.

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Abstract Text

Keywords: Biosensors, Electrophoresis
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Due to its transparent nature, inexpensive fabrication, and ability to reversibly seal to a variety of substrates, polydimethylsiloxane (PDMS) based microfluidic devices have been fabricated for studying biological systems; however, PDMS is not the best substrate for cell studies due to issues with cell adhesion and absorption of hydrophobic molecules. PDMS is also gas permeable, which makes studying gaseous and lipophilic molecules, such as nitric oxide (NO), very difficult to analyze. Therefore, it is crucial to develop more robust microfluidic devices that integrate cell culture and analysis. Polystyrene (PS) has been studied as an alternative microchip substrate, as it is hydrophilic and more biologically compatible than PDMS. A new method of fabricating all-PS devices was developed by etching channels into PS bases. Various bonding techniques (heating, submerging in boiling water, and laminating Shrinky-Dinks) were explored to determine a simple, cheap method to bond two PS substrates. By laminating, there is the ability to make a range of all-PS devices: with and without electrodes and encapsulated tubing. Micrographs of etched channels (straight and intersected channels) were taken using confocal and scanning electron microscopy. Electrophoresis was conducted using a three-sides PS (etched twin-tee pinched channel) and one-side PDMS device. The average plate count was 1,185, with a peak height RSD of 3.8% (n=6). Microchip-based flow injection analysis, with dopamine and NO as analytes, was used to characterize the performance of all-PS devices with embedded tubing and electrodes. Cell immobilization studies were also conducted to assess all-PS devices ability for cellular analysis.

Keywords: Bioanalytical, Electrochemistry, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
This work is carried out as part of the COMMON SENSE European FP7 project. The COMMON SENSE project aims to provide a reliable sensing platform for in-situ measurements on key marine water quality parameters relating to eutrophication, heavy metal contaminants, marine litter and underwater noise. The COMMON SENSE nutrient sensor is based on a combination of microfluidic analytical systems, colorimetric reagent chemistry, low-cost LED-based optical detection, and wireless communications.

The reliable quantification of nutrients in marine environments is challenging due to the low concentration of these solutes in the ocean and the nature of the matrix in which they are held. Initial studies are focussed on validating a method for the sequential determination of nitrite and nitrate in marine environments. Coupled with the traditional well established Griess–Ilosvay reaction for the determination of nitrite, a vanadium chloride (VCl3) solution is used as the reducing agent. The method shows potential as an alternative to the toxic cadmium column for the reduction of NO3- to NO2- in marine water as results indicate that there are no apparent interferences from variances in salinity. The method was tested on a series of samples with varying salinities and sample matrices (costal, estuarine and freshwater), the method is low cost, reproducible and requires low volumes of sample and reagents.
We present a microfluidic breadboard approach for electrophoretic separations based on miniature off-the-shelf components such as syringe pumps, valves, and pressure controllers. For demonstration of its versatility, different configurations with a variety of interlocking components were implemented for very different purposes: capillary zone electrophoresis of cations, the simultaneous separation of cations and anions by dual-capillary zone electrophoresis, the separation of small carboxylic acids by gradient elution moving boundary electrophoresis (GEMBE) and the separation of cationic amino acids by isotachophoresis. Further variations are possible with the breadboard approach. Similar to lab-on-chip devices, the system allows fast separations, as demonstrated by the separation of cations within 35 seconds. On the other hand, it does not require any special manufacturing techniques as it can be assembled from commercial parts, the separation length can be readily adjusted, it is highly versatile and allows the use of superimposed pressures in both directions for added flexibility. The entire system weighs approximately 10 kg, costs less than 15,000 $, and fits into a 25x35x30 cm box. For the automated operation of the whole system, a graphical user interface software was developed, that simultaneously controlled an Arduino microcontroller and a LabSmith electronic interface board. The software was based on the open source package Instrumentino [1], recently developed in our group.

The literature reports shows that trans fatty acids derived from industrial hydrogenation process of vegetable oils is expressed in elaidic acid. Thus, they are included in the nutritional tables, since it comprises 80-100% of trans fatty acids present in processed products that use hydrogenated vegetable fat [1]. The traditional method for fatty acid analysis is gas chromatography (GC). However, in general way, due to laborious step of sample preparations associated to high analysis time (about 60 minutes) necessary to GC approach, alternative methodologies such as capillary electrophoresis (CE) have been considered in the last two decades for different matrixes [3]. Within this context, in the present work, will be presented the general inherent fundamentals and characteristic of cis-trans fatty acids analysis by CE, giving special focus to the total trans fatty acids analysis using direct UV detection.

References:
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Acknowledgements:
Authors acknowledge the financial support of Brazilian foundations CAPES, CNPQ, FAPEMIG and UFJF.

Keywords: Analysis, Capillary Electrophoresis, Food Safety
Application Code: Food Science
Methodology Code: Capillary Electrophoresis
Since its introduction in the early 1980’s, capillary electrophoresis (CE) has become an important separations technique with numerous applications. CE possesses many advantages including high separation efficiencies, fast run times, and small sample requirements. Unfortunately, CE suffers from poor concentration sensitivity and exhibits a low tolerance for matrix components, often necessitating sample processing prior to analysis. Combining separations with mass spectrometry detection has become increasingly critical in many bioanalytical applications. Coupling CE with electrospray ionization (ESI) further reduces salt tolerances and can lower sensitivity via sheath flow, compounding the necessity for sample treatment prior to analysis. Unfortunately, CE still lags considerably behind liquid chromatography (LC) with regards to sample processing, preventing widespread adoption of the technique. Based on the previous limitations of coupling sample processing with CE, a microfluidic platform presents a very promising candidate for an integrated CE system. Microfluidic technology is well suited towards integrating multiple functionalities and can precisely manipulate small volumes with zero dead volume. Furthermore, lab-on-a-chip technologies are amenable to automation, resulting in improved reproducibility and throughput. In this study, we present a novel design coupling microfluidic SPE with microchip CE-ESI with the goal of a fully integrated analysis system combining the sample processing power of LC with the speed and separation performance of CE. The design is reproducible, requires low sample consumption, and has shown a 100-fold improvement in sensitivity while maintaining high separation efficiency and MS compatibility. Furthermore, this design is potentially adaptable to many different applications by utilizing alternate stationary phases, resulting in a fast, high throughput, fully integrated universal platform for sample analysis.
Electrochemical aptamer-based (E-AB) sensors using aptamers as recognition elements have attracted tremendous attention and efforts recently. The specificity, selectivity and high binding affinity provided by aptamer-target interactions coupled with the rapid and sensitive interrogation supported by electrochemical measurements enables a powerful diagnostic tool with rapid response time. However to date, most work demonstrating E-AB sensors is performed using macro-electrodes, thus limiting their ability to be integrated into for example, microfluidic and point-of-care diagnostic systems. Here we report the development of micro/nano-scale E-AB sensors for the detection of ATP with improved analytical performance over existing macrosensors. Specifically, we find that incorporation of nanostructured gold surfaces through deposition of metal nanoparticles onto 25 [micro]m gold electrode creates sensors with higher sensitivity (0.2 vs. 0.04 % signal change/[micro]M ATP) and binding affinity (0.2 [micro]M vs. 0.8 [micro]M) over macroscale sensors. This new small-scale sensor platform is suitable for the development of portable, aptamer-based point-of-care diagnostic devices and will enable the detection of small molecule targets with unprecedented spatiotemporal resolution. We believe this to be a general trend for the development of micro- and nanoscale E-AB sensors.

Keywords: Bioanalytical, Biosensors, Chemically Modified Electrodes, Electrochemistry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Ordered Biomaterials Composite on Glassy Carbon Support as Suitable Platform for Improved Performance of DNA Electrochemical Sensor

The DNA biosensors are powerful tool in bioanalysis. Guanine (G), electrochemical oxidation signal is the most easily observed during DNA oxidation. Changes in G concentrations, the kinetics of electron-transfer reactions or its oxidation potential, are considered indicators of various mutations as well as various interactions with chemical compound. Unbound G oxidation mechanism is complex, complicated and still not well researched.

The structure of the electrode material affects the structure of the adsorbed layer and the nearby solvent. This implies different character of G-electrode surface, G-solvent, and G-intermolecular interactions in the adsorbed layer when changing from one electrode material to the other, and as a result of these variations, also changes in the oxidation kinetics of G may be expected.

Regarding the above-mentioned facts biocompatible and biodegradable materials has been reported as promising and potential materials to overcome the poor and unstable performance of guanine sensor on the bare glassy carbon support, encountered in development of DNA biosensor. Application of various materials consisting of the multiwall carbon nanotubes, the platinum nanoparticles, the mesoporous silica nanoparticles, the hydrogels and the conducting polymers to the construction of sensing layer in guanine biosensors led to the increase the active area by at least order of magnitude compared to the bare glassy carbon support matrix holding guanine. The use of a 3D materials minimize the negative influence of the irregularity in the distribution of the guanine sensing layers and show catalytic effect of oxidation reaction of guanine too. A very good agreement of the data obtained with such independent techniques as electrochemical methods, scanning microscopy and Raman spectroscopy, showed improved electrochemical behaviour of the nowely designed guanine biosensors.

Keywords: Biosensors, Chemically Modified Electrodes, Electrochemistry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Neurons communicate via the processes of releasing and taking in chemical messengers. These neurotransmitters are packaged in nanometer-size synaptic vesicles located in the cell. Quantification of vesicular transmitter content is important in order to study mechanisms of neurotransmission and malfunction in disease and yet this it is incredibly difficult to measure these small quantities in the attoliter volume of a single vesicle. We describe a new approach to electrochemical cytometry to carry out measurements of the total amount of catecholamine transmitters present in individual synaptic vesicles while still in the cytoplasm single cells. We have applied this method to measure the content of vesicles from PC12 cells and have shown the levels in a single dense core vesicle to range from a few thousand to millions of molecules. In addition, these levels can be changed with pharmacological manipulation indicating a new mechanism for discovery of neuroactive drug candidates.
Development of a Photocaged Mecaptan for the Modulation of Hydrogen Peroxide in Brain Slices

Photoremoveable protecting groups, such as p-hydroxyphenylacyl (pHp), have many possible applications in the bioanalytical sciences because of their ability to block the biological activity of a molecule and then restore that activity in a spatially and temporally precise way by application of light. In the past, pHp has been used to protect molecules with very acidic moieties, such as carboxylic acid groups on glutamate or GABA; however, it has not often been used successfully with less acidic moieties such as phenols or sulfhydryls, which are found in a large number of relevant biological compounds. For example, mercaptans, a well-known class of glutathione peroxidase inhibitors, requires a free sulfhydryl group in order to retain its inhibitory activity. As such, it would be advantageous to develop a method to photoprotect this class of compounds at the biologically active sulfhydryl so that hydrogen peroxide levels could be modulated with spatial and temporal precision in tissues. In this work, we synthesized a pHp-MCS ester derivative, protected at the sulfhydryl, and characterized the resulting compound both chemically and photochemically.

Keywords: Bioanalytical, Characterization, Electrochemistry, Neurochemistry

Abstract Text

Bioanalytical, Characterization, Electrochemistry, Neurochemistry

Application Code: Bioanalytical

Methodology Code: Electrochemistry
Room Temperature Ionic Liquids (RTILs) are an exciting class of solvents, receiving much attention in recent years as a replacement for conventional solvents in electrochemical applications. In previous work, we have shown that nanostructures of molecular and RTIL domains were present in mixed solvents. Such heterogeneity has been shown to have significant effect on the electron transfer processes. As of this date, little work has been done on studying the multi-electron transfer of metalloporphyrins in these media. In this investigation, the electrochemical reduction of iron fluorinated porphyrin was carried out in mixed RTILs/organic solvents. The voltammetric analysis showed significant stabilization of the third reduction wave in the mixtures, emphasizing a charge effect of the electrogenerated species. UV-visible spectroelectrochemistry allowed studying the stability of the reduced iron species in the electrolysis timescale. Formation of RTILs/organic domains in mixtures is expected to affect the stability of the reductions products. 19F-NMR analysis allowed the investigation of the effect of such nanostructures on the chemical shifts of the fluoro signals. The occurrence of nano-structural heterogeneity at low concentrations of RTIL in mixtures would obviate the problem of their high viscosity and facilitate their use for electrochemical purposes at a lower expense.
The prevalence of arsenic in ground water and the large number of water sources used for drinking and irrigation makes remote sensor networks highly desirable. The ideal approach should be highly independent of operator intervention; i.e., not require any sort of calibration or blank electrolyte for background correction. To achieve calibration free measurement, a finite volume stopped-flow thin-layer cell (SF-TLC) can exhaustively pre-concentrate dissolved metals in about a minute. The plated metals are subsequently measured by anodic stripping coulometry (ASC). Unlike most previously reported ASC approaches which rely on linear sweep voltammetry or chronopotentiometry, a potential step (PS) ASC variant is utilized to simultaneously strip the deposited metals.

The use of a double PS-ASC also allows in situ blank subtraction. To achieve selectivity, several deposition potentials are used to selectively pre-concentrate a chosen metal. This method has demonstrated measurement of 500 ppb As(III) to better than 10% error even in the presence of high interferent levels (1.3 ppm Cu^{2+}, 500 ppb Cd^{2+}, 500 ppb Pb^{2+}, and 5 ppm Zn^{2+}). Similar performance is possible for As(III) spiked Ohio River water after pH adjustment. This performance is 1-2 orders of magnitude higher than the EPA and WHO recommended limit for arsenic (10 ppb).

This presentation will describe how use of microelectrode arrays (MEA) within the SF-TLC offers improved detection limits. Additionally, MEA design (size and spacing of the individual microelectrodes) will demonstrate that exhaustive pre-concentration within the SF-TLC is possible within about a minute. The combination of these approaches will be demonstrated to allow measurements of less than 10 ppb As(III) in a 2-4 L volume in about two minutes, without any calibration or need for a blank electrolyte and in the presence of other metal interferents.
Heavy metal contamination of the environment caused by increased industrialization is a well-documented, but nonetheless serious problem facing society due to the negative effects these materials have on human health and safety (1). Most analytical techniques require transport of the sample from the field to the laboratory in order to perform analysis, which increases the risk of sample contamination caused by changes in composition. Recently, we have introduced a new methodology for the trace detection of heavy metals: electrochemical x-ray fluorescence (EC-XRF) (2). Here we present an advance of EC-XRF for the real-time identification and quantification of heavy metals (in situ electrochemical x-ray fluorescence). In an in situ EC-XRF experiment, heavy metals are deposited on the surface of a conducting polycrystalline boron-doped diamond electrode while being simultaneously analyzed using x-ray fluorescence spectroscopy. The electrochemical deposition serves as a pre-concentration step, while the XRF measurement provides irrefutable chemical information regarding the identity of the sample. The unique electrochemical cell geometry, which is based on the wall-jet electrode, allows for high rates of mass transport that serve to improve the detection limits of liquid samples compared to conventional XRF (Figure 1). We investigate a variety of parameters on analytical quality, including flow rate, deposition time and potential using Pb as a model system. We also show the possibility of multiple detection using mixed solutions of Pb, Hg, and Cd. The major advance is the potential of the technique to perform analyses in the field without sample pre-treatment or complex reagent addition.

Botanicals are plants or parts of a plant that are used for their flavor, scent or therapeutic properties. The sale and consumption of botanical products has dramatically increased over the past two decades as consumers trend to what are perceived to be natural and high quality botanical products over other manufactured products. The safety of these products, especially products considered to have medicinal or therapeutic properties (i.e. supplements, nutraceuticals or phytomedicines) is often in question. The primary regions of spice and tea production around the world (India, Bangladesh, Turkey, China, and Indonesia) have often been cited as having less stringent safety and quality standards in regards to consumer products. Products from these regions have been studied many times and often have been found to contain a variety of adulterants and contaminants including mycotoxins, pesticides, illegal additives, toxic organic compounds and heavy metals.

In this study, commonly used spices or botanicals in the US (Cinnamon, Mustard Seed, Fenugreek, Ginger, Paprika, Nutmeg, Red Pepper, Black Pepper, Cumin and Turmeric) in various forms and preparations were studied by ICP-MS to determine the presence and level of heavy metal contamination. Many botanical products can often fall into multiple product categories such as spices, teas, condiments and medicinal preparations. Botanical products were selected to cover the range of preparations and uses. Organic varieties when available were also compared to traditionally cultivated botanicals. The botanicals were purchased at a variety of locations including: farmer’s markets, ethnic markets, chain Supermarkets, herbal/health food stores, online and discount stores. Samples were digested using microwave digestion and analyzed using ICP-MS.

Keywords: Contamination, Food Science, ICP-MS, Metals
Application Code: Food Contaminants
Methodology Code: Mass Spectrometry
Abstract Text
The use of dietary supplements is on the increase globally even in the developed countries. Unfortunately, these products are prone to contaminants such as pesticides, microbes, heavy metals, chemical toxins, and common conventional drugs. This observation has triggered interest in their safety. Adelinesuyien et.al. (J. Tradit. Chin. Med. (2013) 33(1): 119-124) detected high levels of Mn (18.545 mg/L) and low levels (<1 mg/L) of other heavy metals (Cu, Cd, Pb, Fe and Zn) in 4 commonly consumed Chinese herbal medicines. Harris et. al. (Sci. Total Environ. (2011) 409: 4297–4305) found that wild plants contained higher levels of heavy metals than cultivated samples. They also found based on assumption of likely mode of consumption that majority of the samples tested contained levels of heavy metals that would not be of concern. However, using conservative scenario of consumption, 69% of the samples contained heavy metals that could contribute to elevated levels of exposure. Given the differences of potential exposure between the ‘likely’ and ‘conservative’ scenarios, the authors recommended more research and monitoring of heavy metals. In our investigations we studied mineral and heavy metal content of three brands of herbal supplements commonly consumed in Indiana. We will report on human exposure to these trace elements based on manufacturer daily recommended intake of the herbal supplements, we will compare this to the recommended daily intakes and use principal component analysis to determine what elements help to cluster them in any pattern.

Keywords: Contamination, Elemental Analysis, Environmental/Biological Samples, Food Contaminants
Application Code: Food Contaminants
Methodology Code: Atomic Spectroscopy/Elemental Analysis
A new analytical method based on slurry sampling combined with a direct hydride generation (HG) for the determination of arsenic and selenium in rice samples was developed. The generated hydrides were introduced to an inductively coupled plasma – atomic emission (ICP-AES) spectrometer. This relatively simple analytical procedure has been shown to give total amounts of arsenic and selenium very close to the results obtained by the determination of these elements after a total decomposition of organic matter and further dissolution of the rice samples.

Some basic figures-of-merit including accuracy and precision of this slurry sampling hydride generation method were additionally studied using a certified reference material. The analytical results were fully comparable.
Near-infrared spectroscopy is a powerful tool for detecting adulteration of food ingredients at economically relevant levels. A single rapid measurement can provide qualitative material identification and quantitative property prediction as well as screening for contamination by undesired materials.

Traditional methods for adulteration screening are either targeted, in which a calibration is built for each adulterant, or non-targeted, in which adulteration is inferred from a poor fit to a model of good-quality material. Both of these methods are valuable, but each has drawbacks.

Targeted methods offer the ability to estimate detection limits and give the best sensitivity, but are time-consuming to develop and are limited to the materials for which they are calibrated. Non-targeted methods do not require calibration for each adulterant, but it is difficult to determine detection limits and the sensitivity is reduced because the method contains no information about the spectral structure of the adulterant.

In this submission, we describe an algorithm that attempts to combine the best elements of targeted and non-targeted approaches while minimizing their shortcomings. Combining a principal components model of the un-adulterated material with a library of adulterant spectra permits much greater sensitivity than SIMCA, but requires only a fraction of the method development time of quantitative methods like PLS.

Keywords: Food Contaminants, Food Safety, Molecular Spectroscopy, Near Infrared
Application Code: Food Contaminants
Methodology Code: Near Infrared
Persistant organic pollutants (POPs) are lipophilic, and can collect in the fatty tissues of living organisms. For this reason, they bioaccumulate through the food chain and can be found in edible oils and oil-containing foods. Testing oily samples for POPs can be problematic due to the high background created by the fats present. Common extraction techniques such as liquid-liquid extraction use nonpolar solvents, which results in a great deal of fatty matrix being coextracted with the analytes. Clean up using large solid phase extraction (SPE) cartridges of silica or alumina is often required for these types of samples prior to chromatographic analysis.

In this presentation, we will present an extraction method for POPs from oily samples that utilizes a new dual-layer SPE cartridge. Specifically, the method will be applied to the extraction of polychlorinated biphenyls (PCBs) from fish and soybean oils, and polynuclear aromatic hydrocarbons (PAHs) from butter. Undiluted sample is weighed directly onto the cartridge, and elution of the analytes is performed in a single step using acetonitrile. The extract is then concentrated and analyzed directly by GC-ECD or GC-MS/SIM. In the case of the fish oil and butter samples, a secondary cleanup was performed using a small silica gel SPE cartridge. The details of the method will be explained, and the presented data will show good recovery and reproducibility of spiked replicates. The proposed method demonstrated advantages in sample cleanup versus other common sample preparation strategies.
The purpose of this study was to develop and validate a method for analysis of pesticides and toxic chemicals in different food matrices using dispersive solid phase extraction. Trace amounts of pesticides and toxic chemicals in different food matrices were determined by modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe), which employed a C18 sorbent, primary secondary amine (PSA), and magnesium sulfate anhydrous in conjunction with a patented compound known as Carbon X for the dispersive solid-phase extraction (dSPE). The carbon X used in this method is similar in design and function to carbon coated silica compounds that better absorb matrix and reduce absorption of analytes of interest. The study was validated on a variety of matrices from vegetables to high fat content foods. Each matrix was fortified with pesticides and toxic chemicals at high and low levels for screening and confirmation, respectively. The extract was then analyzed by gas chromatography-mass spectrometry (GC-MS) or by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Triphenyl phosphate (TPP) was used as internal standard for quantification in both methods. Preliminary data showed promising results with good recoveries and low relative standard deviations obtained for different matrices.

Keywords: Food Contaminants, Gas Chromatography/Mass Spectrometry, Liquid Chromatography/Mass Spectrometry

Application Code: Food Contaminants

Methodology Code: Other
Full-automated high resolution 1H-NMR spectroscopy offers unique screening capabilities for food quality and authenticity by combining non-targeted and targeted screening in one analysis (15 - 20 minutes from acquisition to report). An advantage is its absolute reproducibility and transferability for laboratory to laboratory, which is not equaled by other methods currently used in food analysis. NMR reproducibility allows statistical investigations e.g. for detection of variety, mixing of varieties, geographical origin and adulterations, where smallest changes of many ingredients at the same time must be recorded. Reproducibility and transferability of the solutions shown are user-, instrument- and laboratory-independent. Sample preparation, measurement and processing are based on strict standard operation procedures which are substantial for this fully automated solution. The non-targeted approach to the data allows detecting even unknown deviations, if they are visible in the 1H-NMR spectra of e.g. fruit juice, wine, honey or edible oils. The same data acquired in high throughput mode are also subjected to quantification of multiple compounds. The fully automated 1H-NMR methodology will shortly be introduced and then results on fruit juices, wine and honey will be presented and the advantages of the fully automated 1H-NMR solutions shown. The method has been proven on fruit juices and wine, where so far unknown frauds could be detected. In addition conventional targeted parameters are obtained in the same analysis. This technology has additionally the advantage that NMR is completely quantitative and concentration calibration only has to be done once for all compounds.

Keywords: Beverage, Food Identification, NMR, Quality Control
Application Code: Food Contaminants
Methodology Code: Magnetic Resonance
An immunomagnetic separation (IMS) method was developed for separating Salmonella typhimurium bacteria from large-volume samples of raw eggs. An egg was homogenized with a blender. The homogenized egg material was diluted with a 0.05% Triton X-100 solution to make a 200 mL sample mixture. Anti-Salmonella typhimurium antibody coated magnetic micro particles (MMP) were used to trap the Salmonella bacteria in the sample mixture. The Salmonella-trapped MMP were separated from the egg sample mixture by using a large magnet. An enzyme-linked immunosorbent assay method was adopted and revised for detecting the bacteria trapped onto the MMP. A horseradish peroxidase (HRP) labeled anti-Salmonella typhimurium antibody was used to label the trapped bacteria, and a SureblueTM solution was used as a substrate. The color compound resulted from HRP-catalyzed reaction was detected with UV/Vis absorption spectrometry using a 1 cm sample cell. This simple method can detect $1.4 \times 10^7$ Salmonella typhimurium cells in one raw egg ($7.0 \times 10^4$ Salmonella typhimurium cells/mL in a sample mixture) without any pre-enrichment. The results presented in this paper demonstrate the feasibility of using IMS for separating bacteria from large volume complex samples, which could be adopted for detecting bacteria in other type samples in food safety inspection.

Keywords: Agricultural, Bioanalytical, Biotechnology, Food Safety
Application Code: Agriculture
Methodology Code: Separation Sciences
Determination of traceability in cured ham process is being a difficult task. In this study, we used Synchrotron XAS analysis to direct determination of Fe and Zn species in ham muscle and Zn species in visible fat (intramuscular fat) of Iberian dry-cured ham, IDCH.

Ham samples were obtained from certified IDCH from south of Spain at different curing time ranging from three to forty months. XAS spectra obtained at both Max Lab and ALBA Cells Synchrotrons allow the direct identification of Fe and Zn species in target samples containing low concentrations of Fe and Zn that have not been altered by extraction techniques. Corresponding spectra of individual reference compounds included both organic and inorganic species of Zn(II), Fe(III) and Fe(II) usually present in mammalian meat products.

Results for iron indicate that both organic and inorganic compounds are present in IDCH muscle, three main facts were observed: (1) inorganic iron is always found in less proportion than organic iron, (2) remarkable differences on iron species are found between surface and bulk muscle samples and (3) percentage of Iron (II) species increases with curing time.

With respect to Zn, findings can be summarize as follows: (1) inorganic species were found relatively higher than organic species in IDCH muscle, (2) this difference increases in fat samples, (3) difference between surface and bulk samples are lower than those obtained for iron species. Correlation between results obtained and IDCH curing time will be presented.

Keywords: Food Identification, Metals, Speciation, Spectroscopy
Application Code: Food Identification
Methodology Code: High-Energy Spectroscopy
The level of CO2 inside meat packages is an indicator of freshness. If the CO2 level changes during storage it is a clear indicator that bacteria are growing inside the package and/or the package is not well sealed and the modified atmosphere has been compromised. However, currently there is no available non-destructive way to know the CO2 concentration inside the package (1).

To this end, the objective of SmartPack project is to exploit the development and integration of a CO2 sensor in meat packages using the imaging and communications capabilities of SmartPhones for monitoring the freshness of packaged foods.

An alternative approach to the popular one based on the acidity of the CO2 molecule is employed. The switchable-hydrophilicity solvent, N,N,N′-tributylpentanamidine (TBPA) is used, this molecule is able to reversibly bind CO2 according to the following scheme: TBPA + CO2 + H2O $\rightarrow$ TBPA+ + HCO3-

This reaction involves a large change in the polarity that can be visualised by means of a solvatochromic dye (2). Three solvatochromic dyes have been tested to find the optimum system for CO2 detection.

The performance of the freshness indicator is studied under different conditions, carrying out calibrations at different temperatures and relative humidities using UV-vis spectrophotometry.

Promising results have been found, the next step is to print the CO2 sensor on plastic films currently used for meat packaging and the final goal of this project is to develop the Smartpack App that will allow the CO2 concentration in the package to be tracked simply by taking a picture of the membrane with a smart phone.


Acknowledgements: This research is supported by Talentia Postdoc Fellowship (Junta de Andalucia, EU funded FP7 Marie Curie People, Grant agreement 267226).

Keywords: Food Safety, Gas, Quality Control, Sensors
Application Code: Food Safety
Methodology Code: Sensors
For the food industry, it is important to produce reliable products that are free of hazard and preserve them for long periods. Many microorganisms can grow in food without causing any physical changes like odor, color, texture on food and, at the same time, produce metabolites that threaten human health causing illness and even deaths. New techniques need to be developed for inactivation of molds that cause losses during storage of products and produce the carcinogenic substances called mycotoxins.

In this study, a lab-scale fluidized bed reactor was designed implying a low temperature-atmospheric pressure plasma source and the effect on mold decontamination was investigated. Fluidized bed atmospheric pressure plasma reactor system was characterized based on fluidization velocity, bed height and L/D ratio parameters and the decontamination process was carried out on the model food contaminated in a controlled manner with the aflatoxin-producing mold ([i]Aspergillus flavus, Aspergillus parasiticus[/i]) spores. For this purpose, hazelnut, which have an important place in Turkey's imports was selected as “model food”.

The fungal load on the sample was compared before and after plasma process carried out at variable parameters as treatment time and discharge powers. All the parameters above were optimized so that the performance of reactor was determined.

Atmospheric plasma treatments for 1 and 5 min. resulted in a reduction of [i]A. flavus[/i] population on hazelnuts about 2 and 4.5-log[10] cfu/g, respectively. In the case of [i]A. parasiticus[/i], atmospheric plasma treatments for 1 and 5 min. resulted in a reduction about 2 and 4.2 log[10] cfu/g, respectively.

The background microflora (mold and yeasts) of hazelnuts were treated with atmospheric plasma for 5 min and resulted in a reduction of natural background population to an undetectable level from initial population of about 3.5 log[10] cfu/g.

The alterations occurred after plasma process on morphological structures of [i]A.flavus[/i] and [i]A.parasiticus[/i] spores were demonstrated with Scanning Electron Microscope (SEM).

This study was supported by The Scientific and Technological Research Council of Turkey; Project Number: 113O779

Keywords: Biotechnology, Food Safety, Food Science, Plasma
Application Code: Food Safety
Methodology Code: Other
A Wireless Communication Contact Closure System for Four Mass Spectrometers and Two Liquid Chromatographs in Parallel (LC2/MS4)

Abstract Text
The construction of a wireless communication contact closure signaling system, using newly available electronic components, is described that allows the contact closure start signals from either of two liquid chromatographs to be sent wirelessly to four mass spectrometers and several other detectors, liquid chromatography pumps, and syringe pumps. This new system allows a wide variety of experiments to be performed using two liquid chromatographs, four mass spectrometers, two analog-to-digital converters, an evaporative light scattering detector, a corona charged aerosol detector, and two syringe pumps. Experiments can be reconfigured using only the flips of a few switches to change from multiple parallel mass spectrometry experiments, such as liquid chromatography with 'quadruple parallel mass spectrometry', a so-called LC1/MS4 experiment, to column-switching experiments using two liquid chromatographs and up to four mass spectrometers, LC2/MS4. Additional control of external electronically controlled switching valves involved in column-switching experiments and fraction collection is demonstrated.

Keywords: Automation, Chromatography, Instrumentation, Liquid Chromatography/Mass Spectroscopy
Application Code: Food Science
Methodology Code: Liquid Chromatography/Mass Spectrometry
Direct Mass Spectrometric Screening for Food Contaminants

Directly ionizing foods for mass spectrometric (MS) analysis can be a quick way to screen for contaminants. Techniques, such as DART-MS, can rapidly detect intentional (melamine) or inadvertent food contaminants (photoinitiators) without chromatography or sample preparation. Using direct ionization the entire sample is ionized, yielding a complex MS response. Assigning a signal to an analyte can be difficult since measuring m/z does not provide structural information. Direct MS can even ‘lose’ selectivity by mixing ion signals. Ion fragmentation and high resolution MS (HRMS) can ‘add back’ selectivity/specificity of direct MS, but may diminish the speed or ease of screening for food contaminants. Little experimental data exists demonstrating the adequacy or figures of merit for these direct MS approaches. Similarly, it has not been established whether direct HRMS sample screening will reduce time or effort for food contaminant analysis.

DART-MS was used to screen food, packaging, and cosmetics for targeted (photoinitiators, plasticizers) and untargeted (food contact materials) contaminants. Traditional chromatography-MS analysis (including quantification) of these samples was compared to DART-MS screenings. The effect of DART-MS criteria was examined, including thresholds, and mass accuracy/resolution on accuracy of the direct-MS screening, and the workload. Targeted screening of other food and packaging by DART-MS missed many true positive samples (>20% false negative) when more than two characteristic ions were required to be detected and/or when one of the ions was required to be a pseudo-molecular ion (M+/-X). DART-MS data and screening accuracy for foods, cosmetics, and food packaging will be presented.

Keywords: Food Safety, High Throughput Chemical Analysis, Mass Spectrometry, Validation
Non-nutritive sweeteners are often used in combination in order to limit undesirable tastes such as bitterness. The list of allowed sweeteners varies from country to country due to various health concerns, and new sweeteners are being developed and/or incorporated into foods frequently. Sweeteners are commonly added to beverages, dried fruits and hard candies as alternatives to sugar. There is a need for a multi-sweetener screening technique to save laboratory time and effort. Direct ionization-Mass Spectrometry (direct-MS) techniques allow near instant mass spectrometric (MS) analysis of samples with little or no sample preparation. Direct-MS can identify a wide variety of target analytes, and permits post-hoc analysis for contaminants not originally targeted, making it a quick, flexible screening tool in a busy regulatory environment. Direct ion sources like the DART™ allow samples to be introduced directly into the ionization beam, and resultant high resolution or tandem mass spectra are collected for rapid identification. There is a need for a fast sweetener screening method for the sensitive and selective determination of these analytes in a variety of foods.

In the current study, DART is applied to the identification of 14 sweeteners of regulatory interest in beverages, dried fruits and hard candies. This novel screening method is very useful as a tool for food safety labs to determine if imported and domestic products require additional quantitative/confirmatory analyses. Such a screening technique is not currently available and will allow for rapid assessments of products needing additional testing in order to ensure compliance with U.S. regulations. Data presented here will show efforts to develop a faster, cheaper, DART-MS method to screen a large number of foods for sweeteners.

Keywords: Food Safety, Food Science, Mass Spectrometry, Method Development
Application Code: Food Safety
Methodology Code: Mass Spectrometry
Charge detection mass spectrometry (CDMS) is useful for characterizing the size distribution of large, variable-mass particles such as aerosols, polymers, biomolecules, and even intact cells. The technique relies on the measurement of image charge as highly-charged analyte particles pass near or through a conductor. This is conventionally done using a series of metal tubes. Using a larger number of tubes in series improves the S/N and the accuracy of the charge and mass measurement, although this arrangement is time-consuming to assemble. A much simpler method is to pattern sets of electrodes on printed circuit boards (PCBs). We show that a set of two patterned PCBs arranged as a “sandwich” functions with the same sensitivity as metal tubes. The PCB approach is significantly simpler and less costly to implement.

As a particle passes through an electrode, the image current produced gives the charge on the particle. An arrangement of two such charge detectors separated by a retarding DC potential allows the m/z of the particle to be determined. The measured m/z combined with the measured charge allows calculation of the absolute mass of the particle. We demonstrate that this arrangement of charge detectors and retarding fields can be patterned onto a single PCB device (a two-PCB sandwich). Data are presented for polystyrene microparticles of various sizes and also bacterial spores (B. subtilis).

Keywords: Aerosols/Particulates, Mass Spectrometry, Particle Size and Distribution, Portable Instruments
Application Code: General Interest
Methodology Code: Mass Spectrometry
RNA likely played a key role in the emergence of life due to its abilities to store information, catalyze chemical reactions, and self-replicate; however, it is unclear how the nucleotides were selected and why only four came to dominate modern RNA in life on Earth. In order to study the influences on abiotic polymerization of RNA from monomeric nucleotides, the nucleotides are typically chemically activated, such as with an imidazole group, and allowed to react in the presence of a catalyst such as montmorillonite clay. Our studies indicate that an unactivated nucleotide can be incorporated into the growing strand, most likely terminating the polymerization reaction. This effect is observed only for some nucleotides and not others. A key consideration in these studies is the analysis of the polymerization products. Traditionally, researchers have employed HPLC and gel electrophoresis (GE) to analyze the RNA products. More recently, investigators have also employed MALDI-TOF MS. We are combining MALDI-TOF MS and capillary electrophoresis to study the inclusion of unactivated nucleotides in the polymer products as well as to determine the lengths of the polymer products. Additionally, we are using CE to separate the RNA strands by sequence as well as by length. Using this approach, we hope to obtain more detailed information about the efficiency and chemical selectivity of the polymerization reactions.

Funding source: NASA Astrobiology Grant NNA09DA80A
The RNA World Hypothesis states that RNA was the stepping stone from the prebiotic world to the first life on earth. It hinges on the ability of monomeric ribonucleotides to abiotically polymerize; however, little is known about the processes leading to selection of particular ribonucleotides for inclusion in RNA polymers or their interactions, such as self-assembly, that would modulate their availability for incorporation into growing RNA strands. In addition to the four ribonucleotides in modern RNA, others may have also been included in precursors to modern RNA. One such candidate is inosine monophosphate (IMP) which is of interest because of its likely presence on early earth and its ability to form hydrogen bonds with itself and other ribonucleotides, leading to base pairing as well as formation of alternate structures such as hydrogen-bonded tetrads. Here we describe investigations of IMP monomer that is activated with an imidazole group on the 3' carbon (ImpI) and probe its ability to polymerize with itself and with other unactivated nucleotides (GMP, CMP, AMP, UMP, and IMP) in the presence of montmorillonite clay (MMC), which catalyzes abiotic RNA polymerization, and salts. MALDI-TOF MS is used to analyze the polymerization products from these reactions.

Funding source: NASA Astrobiology Grant NNA09DA80A

Keywords: Characterization, Mass Spectrometry, Nucleic Acids, Time of Flight MS

Application Code: Other

Methodology Code: Mass Spectrometry
Improved Mass Resolution in Microscale Ion Traps for Hand Portable, High Pressure Mass Spectrometry

While mass spectrometers have long been workhorse instruments in laboratory settings, there are numerous field measurements that would benefit from handheld mass spectrometry including safety, security, and environmental monitoring. However, pumping requirements for mass spectrometers have resulted in transportable or fieldable instruments that fall short of handheld forms. Eliminating the turbomolecular pump utilized in these instruments would significantly reduce the system size, weight, and power (SWAP) as well as enhance robustness.

Towards this end, we are developing high-pressure mass spectrometry (HPMS), defined as operation with pressures above 100 mTorr. HPMS reduces pumping requirements through elimination of turbomolecular pumps while enabling instrument platforms with small SWAP. Miniaturized ion traps on the sub-millimeter scale have been used to expand the operating pressure range of the mass analyzer in excess of 1 Torr.

Single CITs and arrays of CITs were fabricated from metal via wet etching with critical dimensions as small as 100 μm. Mass spectra obtained with single CITs, operated at RF drive frequencies up to 60 MHz, displayed unit resolution at operating pressures of 1 Torr of ambient air. CIT arrays demonstrated similar resolution while maintaining the signal intensity of larger traps. To reduce critical dimensions further, CITs were microfabricated in silicon. These silicon-based CITs also demonstrated unit resolution at 1 Torr of ambient air. Further studies into their performance as a function of ambient air pressure will be shown. Through our ion trap development, mass resolution comparable to full scale mass spectrometers is now possible with HPMS.

This project received support from the Defense Threat Reduction Agency-Joint Science and Technology Office for Chemical and Biological Defense.

Keywords: Ion Trap, Mass Spectrometry, Portable Instruments, Volatile Organic Compounds
Application Code: Environmental
Methodology Code: Mass Spectrometry
TO-15 has been around since the 1990s using a mass spectrometer and automated VOC trap pre-concentration system to analyze atmospheric and now soil gas samples using stainless steel canisters. The analysis was initially designed to have detection limits on the low part per billion volume (ppbv) range although many of the risk level concentrations are more than a factor of 10 to 1000 times lower. Performing this method reliably at this low part per trillion volume (pptv) concentration range is challenging and requires very unique approaches to canister cleanliness, calibration, water management and mass spectrometer operating conditions. This presentation will address these issues and suggest the best procedures this laboratory has found to reliably analyze gas samples at pptv concentrations.

Keywords: Air, Chromatography, Environmental/Air, Ultratrace Analysis
Application Code: Environmental
Methodology Code: Mass Spectrometry
Visualization of transdermal drug delivery is important for cosmetic companies that provide topical products containing mild active ingredients. Fluorescent microscopy, CLSM (confocal laser scanning microscopy), and TOF-SIMS (time-of-flight secondary ion mass spectrometry) have been typically used for those purposes. The conventional methods, however, have a distinct disadvantage in which biopsy samples are required to obtain a cross-sectional drug penetration image across skin. We present in this study a novel mass spectrometric visualization method to generate pseudo cross-sectional skin images for fast and non-invasive transdermal drug delivery evaluations. After applying an aliquot of a topical cream containing 1% of 4-methoxy salicylic acid potassium salt (4MSK) to the inner forearm of a healthy male volunteer, skin samples were collected by the tape-stripping (TS) technique. Using the standardized TS procedure, stratum corneum (SC), the most outer barrier layer of epidermis, was collected as a sheet on an adhesive tape. After collecting several SC layers by repetitive TS operations, two-dimensional desorption electrospray ionization / mass spectrometry (2D-DESI MS) was utilized to obtain planer distribution information of 4MS(K) penetrated into SC. The collected data was processed by a patent pending method to create a pseudo cross-sectional skin image showing 4MS(K) penetration through SC layers. Potentials and limitations of the approach will be discussed on the presentation.

**Keywords:** Cosmetic, Electrospray, Imaging, Mass Spectrometry

**Application Code:** Consumer Products

**Methodology Code:** Mass Spectrometry
Histoplasma capsulatum ([i]Hc[/i]) is a dimorphic fungus and causes a respiratory infection known as Histoplasmosis which may develop into a progressive infection, especially for immunocompromised persons. One of the first lines of defense against [i]Hc[/i] is macrophage (M₀), yet [i]Hc[/i] has the ability to avoid immune defenses by replicating within the M₀. One of the body's responses to this infection, using the [i]Hc[/i] strain G217B, is the simultaneous sequestration of Zn and generation of Reactive Oxygen Species through NADPH within M₀ as shown by Vignesh et al. The goal of this work is to determine if [i]Hc[/i] compensates during the immune response by expressing more Zn importers and importing more Cu and incorporating Cu into Zn metalloproteins to maintain function. This information will provide greater understanding into the M₀ host defense mechanism upon infection from the perspective of fungus.

Metal acquisition was examined under altered growing conditions by manipulating the cell media using a chelating resin. Metal profiles were compared through total metal analysis by ICPMS and the proteomic responses were compared using SEC-HPLC-ICPMS. Select chromatographic fractions were collected, further purified by anion exchange chromatography, and then analyzed by tandem mass spectrometry for protein identification. An [i]Hc[/i] database was developed using predicted putative proteins of Hc strain G217B and used to generate a list of predicted metalloproteins using known metal binding domain information. Due to the organism and strain specificity of this database, this application will aid in characterizing the Cu and Zn metalloproteomes of [i]Hc[/i] and the approach could be applied to other microbes. Further work includes quantitative proteomics to compare metalloprotein expression in [i]Hc[/i] under altered growing conditions. Protein targets include Zn importers as well as Cu and Zn proteins selected from HPLC-ICPMS experiments.

Keywords: Bioinformatics, ICP, Liquid Chromatography/Mass Spectroscopy, Proteomics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Mass Spectrometry
Cell surface N-glycoproteins play extremely important roles in cell-cell communication, cell-matrix interactions and reactions with environmental cues. Global analysis is exceptionally challenging because many N-glycoproteins are present at low abundance, such as receptors that only have several copies per cell, and they are membrane proteins with a small portion of them being water soluble and exposed to the outside of cells. Here we have developed a novel strategy integrating metabolic labeling and copper-free click chemistry methods and mass spectrometry-based proteomics techniques to analyze cell surface N-glycoproteins comprehensively and site-specifically. A sugar analogue containing an azido group, N-azidoacetylgalactosamine (GalNAz), was fed to cells to label glycoproteins. Glycoproteins with the functional group on the cell surface were further bound to biotin via copper-free click chemistry under physiological conditions. After protein extraction and digestion, glycopeptides with the specific biotin tag were enriched by NeutrAvidin conjugated beads. The enriched glycopeptides were treated by PNGase F in heavy-oxygen water, and the corresponding N-glycan was removed and asparagine (Asn) was converted to aspartic acid (Asp) and tagged with 18O for MS analysis. In our experiment, we identified 144 unique N-glycopeptides containing 152 unique N-glycosylation sites in 104 proteins in HEK293T cells. As expected, over 90% of identified glycoproteins are membrane proteins, which are highly enriched. Many sites are located on important receptors and transporters on the cell surface. The experimental results demonstrated that the current method combining metabolic labeling, copper-free click chemistry and MS-based proteomics techniques is effective to identify the cell surface N-glycoproteome comprehensively and site-specifically.

**Keywords:** Mass Spectrometry, Method Development, Protein, Proteomics

**Application Code:** Genomics, Proteomics and Other 'Omics

**Methodology Code:** Mass Spectrometry
Monoclonal antibodies are an important class of therapeutic agents that are in widespread use for the effective treatment of many human diseases. We have used a number of mass spectrometers (Synapt G2 and OrbiTrap Exactive EMR) and ion mobility instruments (Synapt G1 modified to an RF-confining drift-cell and a Synapt G2 travelling wave system) to characterise the NIST monoclonal antibody humanised IgG1k molecule, under native-MS and buffer conditions. All glycoforms are resolved on all observable charge states, however only on the OrbiTrap instrument are the glycoforms fully resolved to baseline. Mobility measurements were made in both helium and nitrogen drift-gases on an RF-confining drift-cell device with derived collision cross-section values for charge states +21 to +26 range from 6696 Å² to 6892Å² in helium and 7223Å² to 7403Å² in nitrogen. This small and gradual increase in collision cross-section can be attributed to a combination of increasing ion-induced dipole interaction between the charge protein and the neutral drift-gas and the enlargement of the protein due to the repulsion of the surface charges. The higher charge states (+27 to +29) show a significant amount of gas-phase unfolding. Molecular dynamic simulations on the NIST monoclonal antibody PDB coordinate structure (HOS, Marino) indicate that the gas-phase structure undergoes a significant amount of compaction. Theoretical collision cross-section calculations on the optimised molecular dynamic derived NIST mAb structure are highly consistent with both helium and nitrogen instrument derived collision cross-section values. Collision induced unfolding experiments were also performed on the +25 charge of the NIST monoclonal antibody standard and could potentially be used as a means of providing additional structural information in addition to a single and limiting, gas-phase derived collision cross-section measurement.

Keywords: Biopharmaceutical, Drug Discovery, Protein
Application Code: Pharmaceutical
Methodology Code: Mass Spectrometry
Small molecule neurotransmitters such as dopamine have been implicated in many neurodegenerative diseases, including Parkinson’s Disease and Alzheimer’s Disease. Thus, an understanding of dopamine signaling and distribution is critical. Additionally, in primary neuronal cultures, single-cell measurements have been complicated by the inability to identify cell types prior to measurement. By using a Drosophila line with GFP-expressing dopaminergic neurons and cultivating primary cell cultures, we are able to identify neurons of interest with fluorescence microscopy and perform targeted single-cell analysis on both living and fixed cell cultures. Amperometric measurements were made using carbon-fiber disk microelectrodes to measure potassium-stimulated dopamine release. Dopamine oxidation at the electrode surface results in a current spike that is proportional to the amount of neurotransmitter released and allows for quantification of discrete exocytotic events from live neurons. MALDI-MS measurements were performed using a benzoyl chloride labeling reaction (increasing the sensitivity of this method by an order of magnitude), with a CHCA matrix applied using a commercially available airbrush. Using a ~5 [micro]m diameter laser beam and employing oversampling techniques, we are able to measure neurotransmitters at the subcellular level. This type of measurement at the single cell level has never before been accomplished with dopaminergic cell targets.

**Keywords:** Electrochemistry, Mass Spectrometry, Neurochemistry

**Application Code:** Neurochemistry

**Methodology Code:** Mass Spectrometry
The nervous system is dynamic and heterogeneous in space and chemical content. Determination of often subtle effects of food, exercise and behavior on neurochemical parameters of the brain requires information rich assays. Such assays employ direct mass spectrometry, magnetic resonance spectroscopy and liquid chromatography-mass spectrometry detection. The different requirements for each method lead to compromises such as selecting a single approach or the use of multiple groups of animals to be used. Here we investigate the influence of dietary supplements on neurochemistry of the brain. We used MRI, followed by micro-volume localized magnetic resonance spectroscopy (MRS) of hippocampal brain regions of live anesthetized mice. The same hippocampi are prepared and sequentially analyzed by SIMS imaging, MALDI-MS imaging, and immunohistochemistry. Additionally, 1 mm in diameter punches from the hippocampi are excised and investigated using quantitative LC-MS. These assays produce complementary and, in some cases, overlapping information on hippocampal metabolite composition and localization. For example, GABA and glutamate levels observed in live animals by MRS are verified in LC-MS measurements. Such workflow generated broad metabolite coverage including classical neurotransmitters and lipids, provided distribution information for a number of compounds, and allowed for quantitative measurements of physiologically active molecules both in live animals and in corresponding brain structures after brain extraction.

Supported by Center for Nutrition, Learning, and Memory (UIUC and Abbott Nutrition) grant CNLM-C4219 ZB01.
This work considers the introduction of USP 232/233 the new regulations covering trace metals content of pharmaceutical products[1]. These chapters look at regulatory limits for elemental impurities within pharmaceutical formulations. Issues have arisen over heavy metal content[2] and poor recovery results for the volatile elements from the dated preparation techniques stated in the current elemental impurities USP chapter 231[3]. This also coincides with the recent International Conference on Harmonization (ICH) Q3D regulatory limits for elemental impurities within pharmaceutical regulations. Concern has arisen because of differences in both sets of regulations and how these will be applied to pharmaceutical products. Issues have arisen because of the lack of a consistent analysis method, the lack of a digestion/extraction technique and the lack of an available solid reference material.

The work concentrated on the analysis of the elements As, Cd, Pb, Hg(Class 1) and Mo, Co, Se and V(Class 2A). This work involved the use of a microwave digestion system, (CEM SPD) as a method for tablet preparation and the analytical technique of ICP-MS for sample analysis. The optimisation of a Thermo X Series ICP-MS involved looking at several parameters to determine an optimum set of conditions for analysis based on signal to background ratio. The LoD and LoQ for each element were determined. The technique was applied to standard reference material, excipients and formulated products and the results will be discussed. A comparison of ICP-OES and ICP-MS was made.

References


Keywords: ICP, Metals, Pharmaceutical, Plasma Emission (ICP/MIP/DCP/etc.)
Application Code: Pharmaceutical
Methodology Code: Mass Spectrometry
Base Excision Repair (BER) is one of the essential DNA repair pathways within the body. BER has a focus on non-bulky DNA lesions, such as oxidized or alkylated bases. If the body's BER pathway is dysfunctional, it can lead to gene mutagenesis resulting in several diseases such as various cancers. Many enzymes involved in BER have affinity towards only a small number of lesion types and contexts. However, endonuclease VIII-like 1 (also known as NEIL1) has been shown to act on a variety of substrates, particularly oxidized thymine and guanine bases. NEIL1 exists in two isoforms within the body, differing only in a single residue. Generated by mRNA editing, the edited form of NEIL1 exhibits different substrate specificities and excision efficiencies compared to the unedited form. As the enzyme responsible for the mRNA editing, ADAR1, is upregulated under oxidative stress, it is possible that edited NEIL1 is generated as a response to these conditions. In addition, oxidative stress has been linked to cancer and other diseases in the past, making the levels of NEIL1 isoforms an interesting target for both functional studies as well as a potential cancer biomarker. In this study, NEIL1 isoform levels were determined via affinity purification-mass spectrometry. Isoform levels were compared across healthy cells, cells exposed to oxidative stress, and established breast cancer cell lines.

Keywords: Bioanalytical, Isolation/Purification, Liquid Chromatography/Mass Spectroscopy, Proteomics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Liquid Chromatography/Mass Spectrometry
Presence of multiple peptide isomers in a sample mixture can complicate peptide sequencing and may result in incorrect amino acid sequence matches and/or low sequence coverage scores. Ion mobility (IM) is gaining attention as a rapid separation technique that can be used to separate isomers prior to tandem mass spectrometry (MS/MS) analysis. However, limited IM resolving powers may result in unresolved ion populations. Recently, we demonstrated that “pure” collision-induced dissociation (CID) mass spectra of IM unresolved isomers could be extracted using chemometric deconvolution of post-IM/CID data [J. Am. Soc. Mass Spectrom. 23, 1873 (2012)]. In this presentation, we will demonstrate the utility of post-IM/CID deconvolution to improve sequencing of a four-component mixture of IM unresolved peptide isomers (sequences: RYGGFM, RMFGYG, MFRYGG, and FRMYGG).

Peptides will be mixed at different molar concentrations and ionized using electrospray ionization (ESI) on a traveling-wave IM time-of-flight (TOF) mass spectrometer. Doubly-charged peptide ions (m/z 365.67) will be isolated in the quadrupole mass filter prior to IM separation. After IM, peptide ions will be fragmented using CID and applying a 22 V potential difference between the IM cell exit and entrance of a stacked ring ion guide. Fragment ion masses will be determined using the TOF mass spectrometer. Data will be analyzed using in-house developed automated IM deconvolution (AIMD) software [J. Am. Soc. Mass Spectrom. 25, 1810 (2014)].

A successful deconvolution of CID mass spectra from a four-component peptide isomer mixture is provided in Figure 1. For example, it is shown that deconvoluted CID mass spectrum of FRMYGG matches its corresponding pure isomer which should provide dependable sequencing match scores. Results from de novo database searching software to assess the sequencing scores for IM convoluted peptide mixtures, IM deconvoluted peptide mixtures, and pure peptide isomers will be discussed.

Keywords: Chemometrics, Peptides, Separation Sciences, Tandem Mass Spec
Application Code: Genomics, Proteomics and Other ’Omics
Methodology Code: Mass Spectrometry
Lightweight fiber-reinforced plastics (FRPs) are ideal substitutes for heavier metal constructions in a variety of industries. In particular, carbon fiber-reinforced plastics (CFRPs) are used increasingly in aircraft/aerospace and marine industries as well as the automotive industry. The main objective is to save energy by lowering fuel consumption. In order to guarantee a long lifetime, an FRP has to be tested under various conditions including thermal treatment, mechanical stress and different atmospheres. With DSC (Differential Scanning Calorimetry), the glass transition and the exothermal curing reaction of a thermosetting resin as the polymer matrix for an FRP can be determined and quantified. Cure monitoring by DEA (Dielectric Analysis) is not restricted to a lab environment; it can also be employed for in-process curing of the thermosetting resins and composites. A multi-channel DEA with a fast data acquisition rate can be connected with disposable IDEX (interdigitated electrodes) comb sensors or reusable sensors such as a Tool Mount Sensor. These can be positioned in a mold for resin transfer molding (RTM), or in a press for prepregs or for sheet molding compounds (SMCs), or they are used for infusion processes or in autoclaves at elevated temperatures and pressures. With DMA (Dynamic-Mechanical Analysis) the glass transition, possible post-curing, and stiffness and damping behavior of an FRP specimen can be measured as a function of temperature, time and frequency. An increasingly-used noncontact method for determination of thermal conductivity in and perpendicular to the fiber orientation is LFA (Light/Laser Flash Analysis). Various sample holders and accessories are available for in-plane and through-plane measurements on different geometries of the FRP. The measurement results presented here demonstrate the capabilities of different thermoanalytical and thermophysical techniques for comprehensive thermal characterization of FRPs such as CFRPs.

Abstract Text

Keywords: DSC, Materials Science, Polymers & Plastics, Thermal Analysis
Application Code: Materials Science
Methodology Code: Thermal Analysis
Photoactive azobenzene-containing polymers have generated long-lasting interest and are often investigated using polarized spectral methods. However, the low time resolution of conventional polarized infrared spectroscopy limits its application to slow photoinduced kinetics. To overcome this, we are using polarization modulation infrared structural absorbance spectroscopy (PM-IRSAS), which provides both orientational and conformational information at a 200 ms time resolution, to investigate the photoinduced orientation of both the polymer segments and azobenzene chromophores in thin films of two supramolecular complexes. These complexes are based on hydrogen bonding between poly(4-vinylpyridine) (P4VP) and azobenzene derivatives 4-phenylazophenol (PAP) and 4-hydroxyl-4'-cyanoazobenzene (HCA). Our results show that the in-plane orientation (<P2>) of the azobenzenes decreases with increasing the chromophore content upon irradiation with a polarized 488 nm laser for both P4VP/PAP and P4VP/HCA, the latter having higher <P2>. The residual orientation after the thermal relaxation also decreases with increasing azobenzene content in P4VP/PAP but it increases for P4VP/HCA. The main chain of P4VP barely orients in P4VP/PAP, while photoinduced and residual orientation were only observed for the H-bonded pyridine rings in P4VP/HCA. In this case, the polymer orientation increases with increasing HCA content. Further investigation of this surprising difference in the photoinduced behavior of these two complexes and comparison with birefringence data will also be shown.

Keywords: FTIR, Vibrational Spectroscopy

Application Code: Materials Science

Methodology Code: Vibrational Spectroscopy
Rare Earth Elements (REE) have become indispensable in many electronic, optical, magnetic and catalytic applications due to their specific properties like magnetism and phosphorescence as well as their ability to both donate and accept electrons. Production of high purity REE is now required for advanced technology applications. Therefore, it is important to be able to determine impurity level. In REE mining process, the extracted REE contains many other REE and due to the line-rich spectra emitted by these elements in ICP-OES, the quality of the final result may be compromised. The quality of the results will also be more important when the application involves Nd Fe B magnetic materials. The application of permanent Nd Fe B magnets ranges from hybrid vehicles and wind power generation to handheld wireless devices or computer disc drives. Accuracy of purity determination may also be compromised due to the line-rich spectra emitted by Nd and Fe. This may not only compromise the quality of the material but also related applications. In this presentation, the interest of High Resolution ICP-OES is demonstrated for trace analysis of REE at the ppm level in Cerium oxide and Gadolinium oxide matrices. Results obtained for the determination of trace elements in Nd Fe B magnetic materials are presented. All results and wavelength profiles for both matrices, in addition to recoveries on spiked samples and stability tests are shown.
Finding which samples are similar to each other is a task often encountered in many areas of material analysis, from looking at polymorphs and salt forms in preformulation to determining similar groupings of data in competitive analysis or formulated products. There are many approaches to clustering data and depending on the analysis, there can be different definitions as to what constitutes a similar group.

A significant part of defining various groups involves the analyst’s review of the resulting groups. Once the groups are defined with some confidence, that knowledge can be used to help determine the proper class for new samples. This talk will look at the Euclidean Distance algorithm, some of the limitations in this approach and the data review steps required when clustering data. Examples will include Vibrational, NMR and XPRD data.
Perfluoroalkyl compounds are known to exhibit a hydrophobic character on the material surface, although the C–F bond has a large dipole, which should make the molecular surface polar and hydrophilic. This inconsistency has long been a chemical matter to be solved. In the present study, a stratified dipole-arrays model is proposed: the ‘molecular polar surface’ can fully be hidden by forming a two-dimensional (2D) aggregate of the perfluoroalkyl (Rf) groups, which is spontaneously induced by the dipole-dipole interaction arrays due to the helical structure of the Rf group. On this model, a ‘short’ Rf group should play a role of a single Rf group having a hydrophilic character; whereas a ‘long’ Rf group should spontaneously form a hexagonal aggregate. To examine this model, Rf-introduced myristic acids having various Rf lengths are synthesized, and their aggregation properties are analyzed by using the Langmuir monolayer technique aided by a precise infrared spectroscopic analysis.

Keywords: Characterization, Materials Science, Surface Analysis, Vibrational Spectroscopy

Application Code: Materials Science

Methodology Code: Vibrational Spectroscopy
Material Sciences

Characterization of Liquid Crystal Materials by DSC and TGA-GC-MS

Liquid crystal materials find very broad technical applications. Thermotropic liquid crystals were selected by international organisations for Thermal Analysis (ICTAC, GEFTA, ASTM) for temperature calibration of Differential Scanning Calorimeters (DSC), especially in the cooling mode. In the following paper, typical results are shown for this application. Because of the influences of temperature cycling that were observed, the thermal stability of liquid crystals was tested by Thermogravimetric Analysis (TGA) and the decomposition products were analyzed using a GC-MS directly coupled to the TGA. The selected liquid crystal materials were demonstrated to be well suited for temperature calibration of DSC instruments. The reproducibility of the melting, mesophase transitions, and crystallization depends strongly on the applied temperature limits. The volatile products are identified by coupled GC-MS as fragments of the LC molecules at low temperature, and the molecule ions at higher temperature.

Keywords: Chemical, DSC, Gas Chromatography/Mass Spectrometry, Thermal Analysis

Abstract Text

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Co-Author(s) Ekkehard Post, Erwin Kaisersberger
Our research group has been studying the properties and applications of a carbon material discovered from the pyrolysis of asphalt; GUITAR (Graphite from the University of Idaho Thermolyzed Asphalt Reaction). From various microscopic, spectroscopic and electrochemical characterizations, this material has proven unique and different from other carbon materials, including graphite and graphene. Apart from asphalt, various starting materials including food products have also been successful in the TAR process. Deposition of GUITAR films is chemical vapor based and the formation is conformable to nature and texture of substrate. In this presentation, four major electrochemical properties that distinguish GUITAR from other carbon materials will be discussed. In property (i), the standard heterogeneous electron transfer rate constant across the basal plane (BP) of GUITAR with Ferricyanide of 0.01 cm/s is 2-7 orders of magnitude greater than other BP-graphites. In property (ii), the anodic potential limit of 2.1 volts vs. SHE is 400-700 mV greater than graphites. In property (iii), the hydrogen overpotential in 1 M Sulfuric acid exceeds other graphitic materials by ca. 500 mV. In property (iv), it was determined that the edge planes (EP) of GUITAR are in a chemically different environment than other edge graphitic materials. Based on these properties, application of GUITAR in vanadium redox flow battery will also be discussed.

Keywords: Electrochemistry, Electrodes, Materials Characterization, Voltammetry
Application Code: Materials Science
Methodology Code: Electrochemistry
Analysis of samples with high salt content is common in many application fields. Brine, which is a saturated solution of salt, i.e. a solution containing up to 30% sodium chloride, is used in the food industry for food preservation in both human and animal nutrition. Analysis for heavy metals at trace level in such a solution is then necessary for food safety purposes. Brine analysis is also performed in the chemical industry for the production of products such as chlorine, caustic soda, soda-ash, pure sodium and pure magnesium. It is not only about the quality of the intermediate or final product but also the safety of the process. For example, an excessive amount of magnesium in salt brine of electrolytic cells causes hydrogen evolution that may form an explosive mixture causing damage and also toxic chlorine release. Recently, the exploration of shale oil has led to the need for the analysis of flowback water that may contain high concentrations of salts, chloride and bromide.

Due to the high salt content, the analysis of such samples using ICP-OES may be complex and may imply dilutions, increasing the risk of errors and limiting the sensitivity of the technique. Radial viewing instruments with vertical torch are usually preferred due to their enhanced stability with such samples compared to axial viewing or dual view instruments. However, standard radial instruments have a limited sensitivity for most of these applications.

The performance of a radial viewing ICP-OES with unique features, 3 mm i.d. injector, sheath gas and Total Plasma View, allowing for the measurement of the whole Normal Analytical Zone of the plasma for improved sensitivity is demonstrated in this poster. Results obtained from an undiluted 25% sodium chloride sample are shown with a detection limit in the range of µg/L, as well as the recovery test and long term stability.

**Keywords:** Atomic Spectroscopy, ICP, Spectrophotometry, Spectroscopy

**Application Code:** Materials Science

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
Methods for Bio-Analysis

Low-Cost 3D-Printed Microfluidic Devices with Integrated Electrodes Prepared by Fused Filament Fabrication for Protein Biosensing Applications

3D printing or additive manufacturing has found extensive use in engineering and biotechnology. The impact of 3D printing continues to grow beyond these fields with the emergence of new technologies and materials along with increasing media coverage of 3D printing applications and improving availability of 3D printers. Recently, 3D-printing has been used to make microfluidic devices for chemical mixing, gradient generation, and sensing applications. Here we show that a desktop 3D-printer based on fused filament fabrication (FFF) can be used to prepare microfluidic devices similar to those that have been reported using more expensive 3D-printing methods. We also demonstrate low-cost, easy-to-prepare, 3D-printed microfluidic devices for biosensing based on flow-injection amperometry. Devices are printed using poly(ethylene terephthalate) and feature threaded ports to connect polyetheretherketone (PEEK) tubing via printed fittings prepared from acrylonitrile butadiene styrene (ABS). These devices feature channels designed to have width and height dimensions 800 [µm] and are semitransparent to allow visualization of the solution-filled channels. Electrochemical measurements are conducted in 3D-printed channels by incorporating threaded access ports into which a fitting equipped with electrodes is inserted. We demonstrate that FFF can be used to prepare simple, inexpensive 3D-printed microfluidic devices with integrated electrodes to serve as protein biosensors.

Keywords: Bioanalytical, Biosensors, Electrochemistry, Lab-on-a-Chip/Microfluidics

Application Code: Bioanalytical

Methodology Code: Microfluidics/Lab-on-a-Chip
Methods for Bio-Analysis

Solid-Phase Extraction and Labeling Using a Pressure-Actuated Integrated Microfluidic System

Preterm birth (PTB) is a pregnancy complication that involves delivery before 37 weeks of pregnancy. PTB causes many neonatal deaths and illnesses worldwide. Unfortunately, current clinical methods lack the ability to diagnose PTB at an early stage. Hence, therapeutic interventions are often initiated too late to prevent PTB. Several serum PTB biomarkers have recently been identified,[1] including three peptides and six proteins. We are designing microfluidic-based platforms that integrate immunoaffinity extraction, sample enrichment, on-chip labeling, and microchip electrophoresis for rapid analysis of PTB biomarkers. To date, we have developed a microchip electrophoresis protocol to separate four PTB biomarkers, including three peptides and one protein. Moreover, we have designed a multi-layer integrated microfluidic system consisting of polydimethylsiloxane valves with a peristaltic pump, and a porous polymer monolith in a thermoplastic layer. The valves and pump are fabricated using soft lithography to enable pressure-based sample actuation, as an alternative to electrokinetic operation. We have verified the operation of these pumps and valves. We are presently working to optimize on-chip extraction, enrichment, and labeling in these microdevices. In the future, solid-phase extraction and separation will be integrated to enable rapid, on-chip analysis of PTB biomarkers.

Reference

Acknowledgement
We are grateful to NIH for support of this work (R01 EB006124).

Keywords: Lab-on-a-Chip/Microfluidics, Protein, Sample Preparation, Solid Phase Extraction
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Interactions of soluble proteins with the cell membrane and membrane-bound proteins are critical in regulating the blood coagulation cascade. Among other influences, the membrane lipid composition can greatly affect protein binding and activation; however, a deeper understanding of these effects could lead to new insights that suggest better treatments for bleeding-related conditions such as hemophilia. We have developed a powerful multiplexed assay platform to directly monitor complex protein-lipid interactions by spatially immobilizing phospholipid bilayer Nanodiscs onto silicon photonic microring resonator arrays. Nanodiscs are discrete lipid bilayer mimics that are well-defined by an amphipathic protein belt. They are easily fabricated, offer a high degree of control over lipid composition, and allow for the reliable incorporation of integral membrane proteins. Silicon photonic microring resonators are a label-free and modularly multiplexable sensor technology capable of monitoring binding interactions in real time without the use of enzymatic or fluorescent tags. Nanodiscs containing varying ratios of different lipids (phosphatidylcholine, phosphatidylserine, and phosphatidic acid) were arrayed onto sensor chips. Kinetic rate constants that govern binding and unbinding were then determined for important blood clotting proteins such as activated Factor VII (FVIIa), Factor IX (FIX), Factor X (FX), and Prothrombin. The effects of lipid composition on the kinetic rates of FX and FIX binding to the Tissue Factor (TF)-FVIIa complex were also determined, providing unique insights into a key multi-protein interaction of the blood clotting cascade.

This research was supported by the National Institutes of Health through R01 GM110432.
Non-invasive molecular tests are extremely appealing for cancer diagnosis and treatment where tissue biopsy is highly invasive and costly. Exosomes are cell-derived small membrane vesicles, which are enriched with a subset of molecular components from the origin cells, such as miRNA, mRNA and proteins. Exosomes have been implicated in many biological functions and disease development. However, biology and clinical value of exosomes remain largely unknown, due in part to current technical challenges in rapid isolation, molecular classification and comprehensive analysis of exosomes. Here we have developed a new microfluidics-based approach to improve the isolation and molecular analysis of circulating exosome directly from plasma samples. Our microfluidic technique integrates immunomagnetic isolation and in situ multiplexed immunofluorescence quantification of biomarker expression on exosome surface. Using this method, we demonstrated efficient isolation of CD9+ exosomes directly from plasma of ovarian cancer patients and on-chip quantitative measurement of three tumor biomarkers, CA125, EpCAM and HE4, all streamlined in a rapid assay (<1 hour). Because of the simple design and high performance, this technology holds the potential to accelerate translational investigation and clinical use of exosomes as a non-invasive biomarker for cancer diagnosis.

**Abstract Text**

Non-invasive molecular tests are extremely appealing for cancer diagnosis and treatment where tissue biopsy is highly invasive and costly. Exosomes are cell-derived small membrane vesicles, which are enriched with a subset of molecular components from the origin cells, such as miRNA, mRNA and proteins. Exosomes have been implicated in many biological functions and disease development. However, biology and clinical value of exosomes remain largely unknown, due in part to current technical challenges in rapid isolation, molecular classification and comprehensive analysis of exosomes. Here we have developed a new microfluidics-based approach to improve the isolation and molecular analysis of circulating exosome directly from plasma samples. Our microfluidic technique integrates immunomagnetic isolation and in situ multiplexed immunofluorescence quantification of biomarker expression on exosome surface. Using this method, we demonstrated efficient isolation of CD9+ exosomes directly from plasma of ovarian cancer patients and on-chip quantitative measurement of three tumor biomarkers, CA125, EpCAM and HE4, all streamlined in a rapid assay (<1 hour). Because of the simple design and high performance, this technology holds the potential to accelerate translational investigation and clinical use of exosomes as a non-invasive biomarker for cancer diagnosis.

**Keywords:** Bioanalytical, Biomedical, Biotechnology, Protein

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Many groups have studied organ-level functionality on an in vitro platform by combining microsystem engineering with cell/tissue biology. By creating in vitro models of human tissue (both healthy and diseased) with drug delivery/metabolism, this technique has shown potential to revolutionize human healthcare. 3D printing of such platforms has enabled our group to fabricate bioanalytical systems that are high-throughput, rigid, reusable, reproducible and amenable to automated instruments. In this study, 3D printing was used to fabricate an in vitro platform for studying communication between the endocrine and circulatory systems. Specifically, a pancreatic beta cell mimic (INS-1 cells) was applied to secrete such hormones as insulin and C-peptide into a circulating blood stream, which elicited responses from red blood cells (RBCs) that, in turn, exerted a downstream impact on the vessel barrier mimicked by integrated endothelial cells. It was found that stimulated endocrine release (using 12 mM glucose) increased the release of ATP from $173 \pm 13$ nM to $323 \pm 19$ nM (n=5, error=SEM); downstream on the same device, the RBC-derived ATP stimulated increases in endothelium-derived nitric oxide production from $0.77 \pm 0.10 \text{ M}$ to $1.40 \pm 0.16 \text{ M}$ (n=5, error=SEM). Positive and negative controls can be performed, in parallel, on the same 3D-printed device. Calibration sets are measured in static wells (on the same device) by a plate reader. Importantly, studies of inter-system communication that include a circulation mimic will help create realistic versions of physiological processes using controlled, in vitro platforms.

Keywords: Bioanalytical, Biomedical, Biotechnology, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Methods for Bio-Analysis

Dose Response Study by Creating Concentration Gradient Across 256 Cell Culture Array

A microfluidic diffusion diluter was used to create a stable concentration gradient for dose responses studies. A new calibration method was developed to correct optical artifact effects on the linear gradient. The calibration method was used to find unknown concentrations with 12% error. Flow rate dependant studies showed that changing the flow rates generated different gradient patterns. Mathematical simulations using COMSOL multiphysics were performed to validate experimental data. Experimental data obtained for the flow rate studies agreed with simulation results. Microfluidic diffusion diluter used in this study consisted of 128 culture chambers on each side of the main fluidic channel. Cells can be loaded into culture chambers using vacuum actuation and cultured for long times under low shear stress. Decreasing the size of the culture chambers resulted in faster gradient formation (20 min). Mass transport into side channels of the microfluidic diffusion diluter used in this study is an important factor in creating the gradient using diffusional mixing as a function of the distance. To demonstrate the devices' utility a H2O2 gradient was generated while culturing Ramos cells. Cell viability was assayed at 256 culture chambers, each at a discrete H2O2 concentration. As expected the cell viability for upper side channels increased (inject H2O2) whereas cell viability in lower side channels (inject medium) decreased along the chip due to diffusional mixing as a function of distance. COMSOL simulations were used to identify the effective concentration of H2O2 for cell viability in each side chamber at 45 min. Development of the microfluidic device used in this study will lead to a new direction generating concentration gradients that could be used to study hundreds of concentrations of a drug in a single experiment. In addition, with modification to chip design concentration gradients can be generated on different types of cells in a single chip.

Keywords:
Drug Discovery

Application Code:
Bioanalytical

Methodology Code:
Microfluidics/Lab-on-a-Chip
Parathyroid hormone-related peptide (PTHRP) is a paraneoplastic protein normally expressed in many tissues and recognized as the major causative agent of humoral hypercalcemia of malignancy (HHM). Independent of hypercalcemia, PTHrP has also been implicated in tumor progression and metastasis of a variety of human cancers and its expression mechanistically linked to the development of both skeletal and extra-skeletal metastases. Conventional PTHrP detection methods such as immunoradiometric assay (IRMA) lack the sensitivity required to measure its levels prior to the development of hypercalcemia and pose a health hazard due to the use of reagents labeled with radioactive isotopes. Therefore, there is need to develop ultrasensitive, low-cost assay for PTHrP that can aid in prognosis, diagnosis and therapeutic assessment of cancer patients at earlier stages of the disease and prior to the development of HHM. Here we describe a simple, ultrasensitive modular microfluidic system for on-line capture and detection of different fragments of PTHrP consisting of N-terminal, mid-region and C-terminal peptides. The system features a small microfluidic chamber for on-line capture of the peptides from serum by magnetic beads labeled with many copies of peptide-specific antibodies and signal-transducing enzyme labels. The magnetic bead-peptide conjugate is then washed and directed to a second microfluidic detection chamber housing an 8-electrode array. To reduce the assay cost, gold immunoarrays fabricated by ink-jet printing of 4 nm alkylthiol gold nanoparticles ($0.2) were employed as sensor arrays. Ultralow detection limit of 3 fg/mL (1000 fold lower than the commercially available IRMAs) was achieved for simultaneous detection of PTHrP fragments within a short time (30 minutes). Accuracy and diagnostic utility of the assays was demonstrated by good correlation of the immunoarray level of PTHrP in serum and plasma from cancer patients with results from IRMA.

Keywords: Biosensors, Immunoassay, Lab-on-a-Chip/Microfluidics, Peptides
Application Code: Bioanalytical
Methodology Code: Sensors
Mounting evidence has implicated the excessive production of reactive oxygen species, and its resulting cellular damage, as a key contributor to the development of specific disease states, such as Huntington’s disease and Lou Gehrig’s disease. It is apparent, therefore, that the total antioxidant capacity (TAC), which is directly related to the ability of an organism to combat oxidative stress, is an important parameter to measure in these disorders as well as other conditions. Thus, it is essential to establish a quick, simple, low cost, and sensitive diagnostic method to monitor antioxidant capacity on a regular basis. Microfluidic paper-based analytical devices (µPADs) provide all of these advantages in obtaining such measurements: they are low-cost, easy to use, provide rapid on-site diagnosis, and require low sample volume. In this work, we developed a colorimetric assay on µPADs to measure TAC in blood plasma samples. To run this assay, less than 1-µL of blood is required with subsequent plasma isolation in a unique, flowing plug separation device. Our studies indicate that this method provides good limits of detection of TAC in diluted blood samples. As proof of concept, we measured TAC in blood samples collected from R6/2 Huntington’s disease model mice and their age-matched, wild-type littermates. Our measurements revealed that TAC was significantly greater in the R6/2 sample compared to WT, consistent with the concept that R6/2 mice produce an enhanced capacity to deal with increased oxidative stress. In future studies, this method should allow for rapid diagnosis of biomarkers in blood or other bodily fluids in which only small sample sizes may be available, e.g. cerebral spinal fluid (CSF) or tears.

Keywords: Bioanalytical, Biological Samples, Lab-on-a-Chip/Microfluidics, Neurochemistry
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
The correspondence of chemical bond energy levels (10-100 KCal/mol) with UV/visible frequencies (10^14-10^15 cps) results in a robust spectral resonance that provides a new spectrophotometric technique for the qualitative and quantitative analysis of complex organic substances such as crude oils. Friedel-Crafts (FC) electrophilic alkylations exhibit particularly robust spectral signals in this region while the produced chromophores are adsorbed to the active Lewis acid catalyst, Aluminum Chloride. This strong spectral energy is related to the electronic population inversion achieved in the course of these exothermic chemical reactions as expressed in the definition of a chemical laser as, "a laser operating on a population inversion produced-directly or indirectly-in the course of an exothermic chemical reaction". (Gross, R.W., (1976) "Handbook of Chemical Lasers", John Wiley and Sons. The significance of this new technique lies in the enhancement of the signal-to-noise ratio (SNR) resulting from the strong spectral signals produced when aromatics present in the crude oils (3-30%) undergo the FC reactions forming the chromophores. Thus, a relatively few "marker compounds" present in a complex substance provide definitive identification of the substance. The broadband spectra of these marker compounds undergo multivariate analysis that generates a reference library of standards to which samples are compared. A discussion of the qualitative analysis (curve shape) and quantitative (area under the curve) is presented. The utility of this new chemical reaction/spectrophotometric technique is apparent for the analysis of petroleum hydrocarbons in the oil services industry as well as in environmental applications.

**Keywords:** Energy, Environmental Analysis, Fuels\Energy\Petrochemical, Spectrophotometry

**Application Code:** Fuels, Energy and Petrochemical

**Methodology Code:** Molecular Spectroscopy
Abstract Text
Control and optimization of refinery process units, from distillation, conversion and upgrading units through to final product blending and release, has always been a major concern and objective for refinery operations, critical as it is for effective and economic operation. Process optical spectroscopy analyzers are attractive tools for stream quality measurement. However analytical technologies of this type depend on calibration modelling, correct sample conditioning, suitable installation environment and a dedicated and effective team-work approach to project execution and on-going operational use. This paper will explore these aspects in more detail, and look also at recent technical innovations within process spectroscopy, including simpler more robust process FT-NIR analysers with minimum footprint, utility requirement and significantly extended maintenance intervals, which mitigate some of these costs and allow for low-risk and robust project execution.

Keywords: FTIR, Fuels\Energy\Petrochemical, Near Infrared, Spectroscopy
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Molecular Spectroscopy
The room temperature fluorescence (RTF) quenching of two model PAH compounds was investigated to evaluate the influence of residual water on the interactions between a heavy atom salt (NaI) and the PAH compounds within the novel solid matrix sugar glasses. These interactions have not been thoroughly investigated. Sugar glasses were prepared with a constant analyte concentration and at various quencher concentrations (0.047-0.56 M). The fluorescence intensity was measured with an Agilent Cary Eclipse spectrophotometer. The solvents used to prepare the glasses were mixtures of methanol and water (50/50 and 60/40 respectively). Stern-Volmer plots showed positive deviations indicating a complex type of quenching with glasses prepared with 50/50. Attempts to reduce the static and dynamic quenching components were unsuccessful. However, the quenching sphere of action model of Perrin fit the data; the radiiuses of the quenching sphere for both compounds were determined with values of 10.62 Å for pyrene and 10.12 Å for naphthalene. Reducing the amount of water in the preparation changed the mechanism of quenching to a static ground-state complex type of quenching which was indicated by a linear Stern-Volmer plot with an equilibrium constant of 5.38 ± 0.188 M⁻¹ for pyrene. Residual water left in the sugar glass after preparation influences the interactions of the heavy atom with the fluorophore and could have a significant influence on phosphorescence emission measurements. Reduction of water indicated a more rigid environment by the change in quenching mechanism.

This study was supported by SACP-UARP funding.
Industry today is actively seeking methods of performing more efficiently and using waste streams as alternative sources of income. In many industries the reclaimed food, plant fiber and animal fats are used in a transesterification process to produce glycerol and methyl esters. The fatty acid methyl esters (FAME) produced in the process are commonly used as either a pure biofuel source (B100) or as an additive in diesel fuel. Diesel fuels for automotive applications are commonly blended with FAME and contain biofuel from 2 to 30% (B2-B30). Biofuels are typically transported using various multi-product distribution sources such as pipe lines, tanker trucks, and marine barges that can pose a contamination risk for other fuel sources. For most high performance fuel applications, FAME is a contaminant detrimental to engine performance. As a result of contamination, the issue of FAME in fuels has become a major concern. To analyze FAME in fuels, PerkinElmer has developed an easy to use analysis package containing pre-calibrated quantitation methods for testing of FAME. This analysis technique is based on the European EN 14078 method which uses a liquid transmission cell and Beer’s Law quantitation of the methyl ester carbonyl peak for the analysis of low to high concentrations of FAME. This analysis package has been used to test various fuel samples acquired from commercial distribution sources to determine the FAME content. The results of this testing using pre-calibrated quantitation methods and a portable FTIR spectrometer are presented.

Keywords: Biofuels, FTIR, Fuels\Energy\Petrochemical, Molecular Spectroscopy
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Molecular Spectroscopy
The combination of photoionization spectroscopy and time of flight mass spectrometry has been applied to perform real time studies of tobacco product smoke delivery. The photoionization events come from vacuum ultraviolet photoionization detectors (PID, krypton, 10.8 eV) and a dual-lamp design is implemented to enhance the detection sensitivity. Both the PID lamps and the molecular beam are run in continuous modes while the photo-ions are extracted at 80 KHz into the mass spectrometer. The instrument development enables high detection sensitivity and sub-second time resolution for a puff event, making it possible for the real time measurements of important species produced from smoking, such as glycerin, menthol and nicotine. The measured intra-puff and inter-puff profiles reveal the dynamic evolution of chemicals during smoking.

**Keywords:** Mass Spectrometry, Molecular Spectroscopy, On-line, Volatile Organic Compounds
Infrared and near infrared are useful techniques for QA/QC activities in the pharmaceutical, chemical, petrochemical and food industries. Current FTIR and FTNIRs are highly sensitive, stable and reproducible leading to little or no maintenance for the analysis calibrations. However, deployment of FTIR has been hindered by cumbersome sampling requirements and FTNIR by complicated calibration requirements. In this paper we present a novel disposable sampling methodology suitable for the mid IR that enables easy to calibrate and easy to use QA/QC applications with FTIR.

**Keywords:** Chemometrics, FTIR, Molecular Spectroscopy, Sampling

**Application Code:** Quality/QA/QC

**Methodology Code:** Molecular Spectroscopy
Antioxidants react with the CUPRAC reagent (cupric neocuproine) to produce the Cu(I)-neocuproine (Nc) chromophore measured spectrophotometrically [1]. The method was successfully applied in our laboratory to various food extracts and human serum [2,3]. Lipophilic and hydrophilic antioxidants could be simultaneously assayed in acetone-water as their inclusion complexes with methyl-β-cyclodextrin. A low-cost optical antioxidant sensor (CUPRAC sensor) was developed by immobilizing the Cu(II)-Nc reagent onto a perfluorosulfonate cation-exchange polymer membrane (Nafion®). A novel online HPLC-CUPRAC method was developed for the selective determination of polyphenols in complex plant matrices. Reactive oxygen species such as hydroxyl and superoxide (SO) anion radicals can be detected and their scavenging actions measured by indirect CUPRAC methods, where either the initial probe or its oxidation products showed CUPRAC reactivity. A modified CUPRAC method for measuring the SO scavenging activity of plasma antioxidants (including thiols) utilized a tert-butylhydroquinone (TBHQ) probe with PMS-NADH non-enzymic SO generation system [4]. Recently electroanalytical, QUENCHER, and catalase activity measurement versions of the CUPRAC methodology have been developed. The current direction of CUPRAC methodology can be best described as an integrated train of measurements providing a useful “antioxidant and antiradical assay package” in biochemistry and food chemistry comprising many assays, and the results are in general accordance with those of established reference methods.

References


Keywords: Agricultural, Bioanalytical, Food Identification, Spectrophotometry
Application Code: Food Identification
Methodology Code: Molecular Spectroscopy
UV excitation can provide advantages for Raman measurements because of larger Raman cross sections at short wavelengths, the possibility of resonance enhancement, and the ability to spectrally discriminate fluorescence which occurs at longer wavelengths. We are investigating the use of a spatial heterodyne Raman spectrometer (SHRS) for UV Raman measurements. The SHRS design offers advantages over dispersive Raman systems, especially in the UV, including up to 100 times larger acceptance angle and subsequently a larger field of view, very high light throughput, and high UV spectral resolution in a very small package for a UV spectrometer. The large acceptance angle allows large laser spots to be used on the sample, especially important in the UV to minimize laser-induced sample degradation. In previous work we showed the potential of SHRS for standoff Raman measurements, using 532 nm and 244 nm lasers at 5 meter standoff distance. In recent work, standoff UV Raman measurements were made using an SHRS with a 266-nm pulsed laser and a gated detector at standoff distances of ~20 meters, in bright ambient light conditions. In this paper a brief description of both benchtop and standoff UV-SHRS systems will be given and the overall system performance will be discussed. Key performance issues will be highlighted and applications described with emphasis on space exploration and planetary measurements.
Nonpolar phase synthesized hydrophobic nanocrystals show attractive properties and have demonstrated prominent potential in biomedical applications. However, the preparation of biocompatible nanocrystal is made difficult by the presence of hydrophobic surfactant stabilizer on their surface. To address this obstacle, we have developed a facile, high efficiency, single-phase and low-cost method to convert hydrophobic magnetic nanoparticles (MNPs) to an aqueous phase using tetrahydrofuran, NaOH and 3, 4-dihydroxyhydrocinnamic acid without any complicated organic synthesis. The as-transferred hydrophilic MNPs are water soluble over a wide pH range (pH=3 to 12), and the solubility is pH-controllable. Furthermore, the as-transferred MNPs with carboxylate can be readily adapted with further surface functionalization, varying from small molecule dyes to oligonucleotides and even enzymes. Finally, the strategy developed here can easily be extended to other types of hydrophobic nanoparticles to facilitate biomedical applications of nanomaterials.

Keywords: Biotechnology
Application Code: Biomedical
Methodology Code: Other
To date, quantification of inorganic nanoparticles in cells has been limited to bulk analysis of homogenized cell samples typically by elemental analysis methods such as inductively coupled plasma mass spectrometry (ICP-MS). These methods provide an average concentration per cell; not ideal for understanding the heterogeneity of the nanoparticle concentration per cell within cell populations. This study investigates synchrotron X-ray fluorescence (SXRF) microscopy, a promising tool to quantify elements in cells, to quantify and characterize the distribution of titanium dioxide nanosphere uptake across cell population. For this study, individual cells were scanned using a dwell time of 5 seconds per pixel and spectral summing in the area around the cell was used to get counts of Ti within the cells. These counts were used with NIST standard 1833 for titanium to quantify the Ti concentration per cell. These results were compared with average nanoparticle concentrations per cell obtained by widely used ICP-MS. The results show that nanoparticle concentrations per cell quantified by SXRF were of one to two orders of magnitude greater compared with ICP-MS. The SXRF results from 112 cells also indicate a Gaussian distribution of the nanoparticle concentration per cell with internalized nanoparticle concentrations from 773–1751 pmol. The results suggest that quantification of nanoparticles in cell populations is subject to limitations of bulk analysis methods including potentially misleading information from taking large population averages to determine physical parameters and the lack of any information about the cellular heterogeneity.

Acknowledgement: This work was supported by the National Institute of Health (grant No. 1DP2OD007394-01).
Bioanalytical Applications of Single Nanoparticle Collision at Microelectrodes

Single nanoparticle collision based electrochemical methods have demonstrated significant promise for detection and quantification of nanoparticles. In this presentation, we describe development of an electrochemical collision technique to characterize the fundamental surface properties, functionalization and reactivity of metal and metal oxide nanoparticles by nano-impact electrochemistry as the particle collide with a microelectrode. We will demonstrate several applications of this method for assessment of redox active nanoparticles, for monitoring biorecognition events at single particle surfaces for applications in sensing, and as a method enabling rapid quantification of the extent of surface attachment of bioreagents on individual nanoparticles. We will discuss the potential of this approach to complement or replace costly characterization techniques and enable routine study of nanoparticles and their reactivity.

Keywords: Bioanalytical, Electrochemistry, Nanotechnology, Voltammetry
Application Code: Biomedical
Methodology Code: Electrochemistry
Cells in the human body are constantly receiving and integrating biomolecular and mechanical cues in a spatially defined manner. Establishing the correlation between these signals and the resulting cellular responses is a driving force in biomaterial design; however, it remains a challenge to orthogonally control mechanical and biomolecular cues. This study presents an approach for spatially controlled, overlapping growth factor immobilization using benzophenone photolithography on collagen-glycosaminoglycan membranes. Controlled material stiffness independent of biomolecule immobilization was achieved using carbodiimide crosslinking. We examined the influence of mechanical and biochemical properties on adipose-derived mesenchymal stem cell bioactivity and lineage-specific gene expression. Results revealed that bone morphogenic protein (BMP-2) and platelet derived growth factor (PDGF-BB) significantly influenced stem cell fate on softer materials, while mechanical properties dominated lineage commitment on the stiffest materials. The described approach enables us to more closely mimic the native extracellular matrix as a means of systematically investigating cell behavior. Further efforts are focused on strategies for dynamic tuning of chemical properties to enable spatial and temporal manipulation of cellular microenvironments.
Identification of proteins at low abundance or with post-translational modifications is important in the biomedical and clinical research. We propose the microfluidic chips or mesoporous materials as bioreactors for highly efficient and controllable analysis of low-abundant proteins, phosphoproteins, glycoproteins and membrane proteins. A droplet-based microreactor is developed for on-line proteolysis reaction and also acts as an interface to fractionate proteins separated by liquid chromatography and identification by ESI-MS/MS or MALDI-MS. A facile online enrichment protocol is proposed based on microfluidic droplets, and low-abundance biological species are successfully concentrated and analyzed in such a system via the droplet shrinkage. Additionally, the surface interaction or surface charge controlled reaction exhibits highly proteolytic efficiency due to the in situ adsorption of different proteins from bulk solutions into the nanopores or microchannels, where the target biomolecules and enzymes are selectively confined in the microspace to realize a quick biological reaction. The ordered mesoporous matrices tailored by different pore size, surface charge and functional group modifications have been used as nanoreactors to favor the rapid protein digestion and analysis, where the reaction kinetics is simulated.

Keywords: Lab-on-a-Chip/Microfluidics, Liquid Chromatography/Mass Spectroscopy, Nanotechnology, Proteomics
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Appropriate physical-chemical characterization to determine product quality is vitally important during pre-clinical testing of drug products containing nanomaterials. As a case study, gold, silver, and silica nanoparticles with different physical-chemical attributes and stability considerations were followed through an 8 week repeat dosing study. Particle size and size distribution, morphology and agglomeration, zeta potential, pH, concentration, purity, chemical composition, endotoxin burden, and particle stability in the dosing solution and rodent serum were determined for the stock solutions at the initiation of the study. Particle size and size distribution, pH, agglomeration status, and concentration were then evaluated each week after formulation and post animal dosing. Instrumentation used for the characterization included dynamic light scattering, transmission electron microscopy, UV-vis spectroscopy, and neutron activation analysis.

The silica and gold nanoparticles did not have appreciable stability issues over the dosing timeframe. Silver nanoparticles showed particle instability as well as sensitivity to the formulation and dosing environment as evidenced by agglomeration within the formulation containers and syringes. Interestingly, when reporting the simple numerical results such as Zave or average Feret diameter, there was no evidence of instability, even when the agglomeration and plating was so extensive that it could be observed visually. Histograms obtained by dynamic light scattering as well as morphological analysis of transmission electron micrographs indicated the instability of the formulation. This result highlights the importance of understanding the critical quality attributes of the nanoparticle formulation, the correct methodology to measure these attributes, and the correct ways to analyze the data arising from the methods. By having a solid understanding of these issues, the quality of the product is more likely to be ensured.
This research aims at developing a high throughput biosensor for protein detection based on the nanohole array technology and a custom-made 96-well plate reader. Gold nanohole arrays exhibit distinct plasmonics properties depending on the excitation mode of the surface plasmon polaritons (SPPs). Excitation of such plasmonic material requires simple optical setup working in transmission spectroscopy, benefit from the availability of low-cost manufacturing technologies and exhibit high sensitivity. Gold nanohole arrays can be excited in SPR with the Kretschmann or by grating coupled attenuated transmission (GCAT) configuration. Kretschmann configuration leads to higher sensitivity in nanohole arrays. Tuning the excitation conditions in GCAT can narrow this gap in sensitivity for nanohole arrays between both modes of excitation. Thereby, the optimization of the excitation angle of gold nanohole arrays in GCAT mode leads to an improvement of the sensitivity to binding events. To prove this, we performed a study of the surface and bulk sensitivity of the (1,0) Bloch-Wave SPPs mode for gold nanohole arrays 1000 nm of periodicity for an excitation angle range from 0° to 30° from the normal. At an optimal angle, the wave length shift, in comparison to direct transmission analysis, for the detection of IgG (limit of detection of 1 nM) was improved by a factor of 3. Optimization of the excitation angle of nanohole arrays affect the confinement of the plasmonic field which can lead to applications with techniques such as surface enhanced Raman spectroscopy (SERS) and metal enhanced fluorescence (MEF). Thus, a 96-well plate reader was designed to analyze nanohole arrays at different angles. In conclusion, gold nanohole arrays hold great promise for the development of a future multiplexed plasmonic transducer.

Keywords: Bioanalytical, Biosensors, Nanotechnology, Sensors
Application Code: Bioanalytical
Methodology Code: Sensors
Measuring proteomic biomarkers which are shed into the bloodstream during disease progression serve as non-invasive mode for clinical diagnostic. However, the greatest challenge in their detection is their rarity in the blood ($10^{-18}$ to $10^{-12}$ M). In the present work, a technology platform is introduced that integrates Surface Plasmon Resonance imaging (SPRi) and aptamer technology with nanomaterials and microwave-assisted surface functionalization. This unique combination and integration makes it possible for the SPRi biosensor to detect C-reactive protein (biomarker) in spiked human serum at attomolar ($10^{-18}$ Molar) level (zeptomole or fg/ml). The results are encouraging and show promise in extending the platform to detect an array of biomarkers in complex biological fluids that are indicative and even predictive, of disease onset and disease progression.

Keywords: Biosensors, Nanotechnology, Protein
Application Code: Biomedical
Methodology Code: Biospectroscopy
While one of the most basic steps in chromatography, proper column installation can make or break chromatographic system performance and productivity. Forming and maintaining leak free connections is critical in achieving low background noise, sharp peaks, optimal column resolution and lifetime. Small leaks, excess void volume and flow path activity take their toll on chromatographic results regardless of the mobile phase being used.

Chromatographers working with HPLC and UHPLC are often challenged by problems such as peak tailing, peak broadening, split peaks, carryover, etc. One common cause for these problems that is often overlooked, and time consuming for troubleshooting, is bad tubing connections. Dead volume or micro-leakage in tubing connections can strongly affect the performance and reproducibility of HPLC and UHPLC analysis, especially with ultra-high pressures and small column dimensions.

Chromatographers working with GC and GC/MS systems often need to retighten fittings as the materials in some ferrules shrink with repeated heat cycling. A new innovative self-tightening column nut eliminates the need to retighten these fittings that chromatographers have learned to live with over the years. These new fittings coupled with a suite of deactivated connectors for gas phase applications make forming and maintaining gas tight connections easy and reliable.

**Abstract Text**

While one of the most basic steps in chromatography, proper column installation can make or break chromatographic system performance and productivity. Forming and maintaining leak free connections is critical in achieving low background noise, sharp peaks, optimal column resolution and lifetime. Small leaks, excess void volume and flow path activity take their toll on chromatographic results regardless of the mobile phase being used.

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**Keywords:** Gas Chromatography, Gas Chromatography/Mass Spectrometry, High Throughput Chemical Analysis, Other

**Application Code:** Other

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Comprehensive two-dimensional gas chromatography (GCxGC) is the most effective technique for the analysis of complex volatile and semi-volatile mixtures. A single-stage thermal modulator for GCxGC that requires no consumables for operation has been developed that traps, focuses and reinjects analytes into the second column using a specially prepared coated stainless steel capillary trap. Desorption is completed using a capacitive discharge power supply to resistively heat the trap, while rapid cooling is accomplished by compressing the steel capillary between two passively or actively cooled ceramic pads. An evaluation has been completed that focuses on the repeatability and reproducibility of the results obtained with the device. Investigated samples included diesel fuel, a fragrance mixture, and environmental pollutants including polychlorinated biphenyls, organochlorine pesticides and chlorobenzenes. Replicate analyses of these samples have been performed and within-day repeatability, day-to-day reproducibility, as well as between-trap reproducibility were assessed. The results are very encouraging as negligible shifts in retention times were observed for both within-day and day-to-day comparisons of the studied samples. Eliminating retention time shifts vastly simplifies the advanced statistical analysis of data without the need for alignment procedures. Routine analysis and quality control applications will also benefit from improved retention time reproducibility. An overview of the device operation and the results from this study will be presented.

Keywords: GC, Instrumentation, PCB's, Petrochemical
Application Code: Quality/QA/QC
Methodology Code: Separation Sciences
Structure determination of proteins of various origins is focus of studies by numerous instrumental methods. Specific behavior of these macromolecules consisting of amino acid residues under mass spectrometry conditions can be interpreted on the basis of established fragmentation pathways of the initial amino acids. A systematic study of alkyl and/or perfluoroacyl derivatives of amino acids and their $^{2}$H-, $^{13}$C-, $^{15}$N-analogs by GC-MS has been carried out for the first time in this research. Mass spectra of L-glycine, L-alanine and L-phenylalanine derivatives will be used for establishing general fragmentation patterns under electron ionization conditions. Among specific dissociation directions the following topics will be discussed: (a) diagnostically important cations useful for structure determination of L-valine, L-leucine and L-isoleucine derivatives, (b) competing fragmentation processes in the molecules of substituted L-serine, L-threonine, L-cysteine, L-methionine, L-aspartic and L-glutamic acids, and (c) charge localization under electron ionization dissociation of substituted L-lysines as well as L-prolines and L-tyrosines. These specific fragmentation pathways for most amino acid derivatives can be useful for their structure determination within peptides and proteins. A special attention will be paid to the formation of nitrilium cations containing 2-C and amino N-atoms, and to ions due to oxygen and hydrogen rearrangements; the mechanisms will be discussed.

**Keywords:** Amino Acids, Derivatization, Gas Chromatography/Mass Spectrometry

**Application Code:** Bioanalytical

**Methodology Code:** Gas Chromatography/Mass Spectrometry
The US Environmental Protection Agency (US EPA) is currently revising Methods 8260 and 8270, which are commonly used gas chromatography/mass spectrometry reference methods for analysis of volatile and semi-volatile organic chemicals in environmental matrices. These methods are part of the SW-846 compendium of Test Methods for Evaluating Solid Waste which provides test procedures and guidance for use in conducting the evaluations and measurements needed to comply with the Resource Conservation and Recovery Act (RCRA) [superscript 1]. This presentation will address the major changes to each of these methods, including the addition of the lower limit of quantitation (LLOQ) verification, updates to the performance data and appropriate preparation techniques, expansion of the analyte lists (to include those frequently found in Superfund sites), addition of advanced mass spectrometry techniques (Tandem Mass Spectrometry, Time-of-Flight), guidance for using hydrogen carrier gas, and clarification of requirements for some quality control elements. This presentation will also discuss the harmonization of these methods with each other, and where possible, harmonization with waste water Methods 624 and 625. This presentation will provide an opportunity for attendees to see the revisions to the methods. These validated methods are expected to be posted on the RCRA Methods website [superscript 2] for comments and use in 2015, with finalization to follow.
In addition to traditional applications performed by the classical 624 type stationary phase, the DB-624UI was found suitable for use in a number of difficult and challenging chromatographic applications like the characterization of oxygenated compounds in waste water, phenol and alkylated phenols used as anti-oxidant in fuels and lubricants, furans analysis for cellulose degradation monitoring, and sulfur compounds in hydrocarbons to name a few. Example chromatograms highlighting these promising results will be shown.

The stationary phase demonstrated a high degree of inertness with low bleed characteristics with a maximum operating temperature of up to 260°C and a unique selectivity. The column is available in different dimensions and was found to be compatible with different GC techniques such as single or multi-dimensional gas chromatography, comprehensive two-dimensional gas chromatography and hyphenated techniques like GC-MS.

Keywords: Capillary GC, Environmental Analysis, Fuels\Energy\Petrochemical, Oxygenates
Application Code: Process Analytical Chemistry
Methodology Code: Gas Chromatography
We combined an Open Probe inlet with a low thermal mass (LTM) ultra fast gas chromatograph (GC) and a mass spectrometer (MS) of GC-MS, forming a new method and device for obtaining real time analysis with separation. The Open Probe is based on a vaporization oven that is open to ambient pressure while having helium purge flow protection to eliminate air leakage into the oven and MS ion source.

Sample introduction into the Open Probe is as simple as: touch the sample, insert the sample holder into the open probe oven for its thermal vaporization and have the mass chromatogram data in 20-30 s.

The Open Probe is mounted onto the LTM fast GC that is coupled either with the Aviv Analytical 5975-SMB GC-MS with Cold EI, or with the Agilent 5975/5977 MSD for obtaining in-vacuum electron ionization, followed by quadrupole-based mass analysis. Open Probe fast GC-MS provides real time analysis in combination with GC separation, library identification, absence of ion suppression effects and uniform electron ionization response for improved quantitation. Furthermore, it employs the simple and low cost mass spectrometer of GC-MS.

The operation of the Open Probe fast GC-MS will be demonstrated with forensic application including:

- 30 s full analysis cycle time of heroin in its street drug powder.
- Fast THC and CBD analysis in Cannabis.
- Cockroach repeller liquid residue on tomato analysis in 30 s.
- Trace TNT on human hand that was analyzed in 40 s with NIST library identification of TNT.

Keywords: Forensics, Gas Chromatography/Mass Spectrometry, Mass Spectrometry
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
Techniques in Forensic Analysis

The Influence of Temperature and Atmospheric Composition on the Pyrolysis of Household Materials

Fire investigation is always a challenging task for forensic chemists. Recently, we have demonstrated the application of chemometric tools to the automated identification of gasoline in casework arson samples. In order to expand these methods to a wider library of ignitable liquids (ILs), a method to simulate fire debris would be ideal. However, established simulation methods have been shown to be inadequate for the purposes of training a model to classify casework arson samples.

Here, we present the results of a study into the effects of pyrolysis conditions on the pattern of observed pyrolysates when analysed by GC in order to have a better understanding of pyrolysis and to ultimately enable the rapid generation of fire debris suitable for training chemometric models for casework samples. A variety of substrates were pyrolysed individually and in various combinations. Parameters tested for the pyrolysis include the pyrolysis temperature, the heating profile for the samples, and the composition of the atmosphere in the pyrolysis tube.

Keywords: Chemometrics, Forensics, Gas Chromatography/Mass Spectrometry, Pyrolysis

Application Code: Homeland Security/Forensics

Methodology Code: Gas Chromatography/Mass Spectrometry
Synthetic cathinones are a class of psychoactive compounds marketed as alternatives to drugs like methamphetamine and ecstasy. Cathinones are addictive and their use has been linked to overdoses and deaths. Although legislation has attempted to restrict these compounds, only a few specific compounds are legislated while others have indeterminate legal status. With new derivatives of cathinones continually surfacing, controlling these substances is challenging.

Ambient MS methods increase throughput of analyses as well as provide more detailed structural information of unknowns. DART-MS is an ambient technique where an unknown white powder can be ionized directly without extraction or chromatography, greatly reducing analysis time. As a preliminary screening tool, DART-TOF-MS has the potential to reduce backlogs and streamline analysis.

DART-MS was used to characterize individual cathinones and mixtures of cathinones and various cutting agents. Simple spectra can provide parent [M+H]+ peaks indicative of the number of compounds present, with high mass accuracy key in predicting formula weights. Cathinones were also fragmented by in-source collision induced dissociation (CID) to produce fragment patterns for structural characterization, used to differentiate isobaric cathinones based on characteristic fragments. Furthermore, a mixture of cathinones shows that individual components can be distinguished within a mixture. The process allows for rapid identification of common cutting agents, providing a means to simplify more complex mixtures. With no sample preparation needed, spectra were obtained in seconds, and the DART-MS is ideal for structural characterization of unknowns.

Finally, DART-MS data is suitable for statistical analysis. Any differences in samples due to degradation or many other factors can be the basis of statistical differentiation against several statistical models and programs, allowing the reporting of results with a higher level of confidence.

Keywords: Forensic Chemistry, Mass Spectrometry, Statistical Data Analysis
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
Morphologically Directed Raman Spectroscopy (MDRS) can be applied to a variety of forensic evidence types, such as illicit drugs, counterfeit pharmaceuticals, hoax powders, soils and gunshot residues. It is a novel and reliable tool that would enable criminalists to obtain more information from forensic samples than is currently employed for investigations and adjudications. MDRS combines automated particle imaging and Raman spectroscopy in one instrument. Particle imaging is performed to determine particle size and shape distributions of components in a blended sample. Particle size is an important physical property of particulate samples because it has a direct influence on a variety of material properties such as reactivity or dissolution rate, suspension stability, efficacy of delivery, texture, feel, appearance, flowability, handling, viscosity, packing density and porosity. Although measurement of particle size distributions is routinely carried out across a wide range of industries and is often a critical parameter in the manufacture and analysis of many products and substances, it is not widely used in the forensic sciences. Raman spectroscopy is a useful technique in forensic science for determining molecular chemistry because it is rapid, reliable, does not require contact with the sample, and is non-destructive. Combining these two analytical techniques allows the individual components present within a blend or mixture to be independently characterized and compared. This presentation will demonstrate how such a tool can be used to gain a better understanding of mixtures across many areas of forensic science, as it is applicable to a range of Raman active samples.
Methamphetamine is a highly addictive drug and its manufacture, distribution, and abuse are issues of international importance. Enforcement agencies continue to develop methods to characterize methamphetamine samples. In particular, significant efforts have been directed to investigation of the chemical signatures that are characteristic of the starting material and methods used for its synthesis, which can be used to track the activities of clandestine laboratories, distribution networks, and trafficking patterns. These chemical signatures include trace impurities such as residual starting materials and/or by-products from the synthetic method. Methamphetamine was prepared from five different synthetic routes and characterized using direct analysis in real time mass spectrometry (DART-MS). DART-MS is an ambient ionization method that was used to characterize samples in their native form without any preparation. In addition to methamphetamine, dozens of different impurities were identified by accurate mass measurements. These impurities included residual starting material, intermediate products, route-specific identifiers, and other synthetic by-products. Together, these various impurities serve as a specific chemical signature for methamphetamine that is related to the synthetic method and starting materials employed. The DART-MS method used here demonstrates the utility of the technique to rapidly characterize methamphetamine samples in a manner different, yet complementary to current standard methods.

Keywords: Drugs, Forensics, Forensic Chemistry, Mass Spectrometry
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
Blood stains, which are among the traces encountered most frequently at crime scenes, are important for potential extraction and amplification of DNA for suspect identification, as well as for spatter pattern analysis to reveal a sequence of events. Determination of blood stain age can contribute to verify witness’ statements, limit the number of suspects and confirm alibis. As a result, estimate the age of blood stains with good accuracy and precision has been an elusive goal for forensic investigations.

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was used in forensic detection of blood stains and age estimation because of signature absorbances in the mid-infrared region at 3300 cm$^{-1}$ (Amide A), 2800 cm$^{-1}$ to 3000 cm$^{-1}$ (Amide B), ~1650 cm$^{-1}$ (Amide I), ~1540 cm$^{-1}$ (Amide II), and 1200 cm$^{-1}$ to 1350 cm$^{-1}$ (Amide III). Peak position and intensity shifts of blood spectra due to aging changes occurring as a result of the denaturation of blood proteins and water absorption/desorption were observed with FTIR. Principal component analysis (PCA) and partial least square regression (PLS) were used in this work to combine these changes in a multivariate calibration that reveals correlations between the amide peak changes and blood age. Calibration experiments over several months at 30 degree Celsius under a variety of humidity and substrate conditions enable prediction of blood stain age under different environmental conditions.

The results contribute to the understanding of mechanisms of blood aging and provide a nondestructive approach for predicting blood age under a variety of environmental conditions.

Keywords: Bioanalytical, Chemometrics, Forensic Chemistry, FTIR
Application Code: Homeland Security/Forensics
Methodology Code: Data Analysis and Manipulation
This report reveals the potential of enhancing forensic fingerprint evidence by adding a qualitative chemical dimension. The chemical residue on the skin of the person leaving the fingerprint on a reflective surface may potentially be identified by infrared microspectroscopic techniques. Various commercial skin lotions and hair preparations have an organic component in their formulation that enables differentiation according to the brand. A partial library of spectra from a clean reflective substrate surface is used for comparison of the incidental fingerprint residue deposited on an everyday reflective item. Using the background obtained from an organic coating in proximity of the fingerprint, the spectrum of the surface deposition is obtained.

Keywords: Forensics, Infrared and Raman, Microspectroscopy, Trace Analysis
Application Code: Homeland Security/Forensics
Methodology Code: Vibrational Spectroscopy
A compact, rugged wide-field electronically tunable wavelength filter has been developed for hyperspectral imaging applications in the visible and near-infrared region. The filter employs surface plasmon coupling in metal films separated by a tunable dielectric layer. The advantages of the surface plasmon-coupled filter (SPCF) are its large spurious free spectral range (450nm-1000nm), high throughput (>50%), and compact design. The rugged optical assembly, ease of operation, and small size make it suitable for hand-held hyperspectral imagers as well as for incorporation into existing optical assemblies. Unlike conventional electronically tunable widefield imaging filters such as the acousto-optic tunable filter and liquid crystal tunable filters, the SPCF provides a straight-through optical path without the need for intermediate polarizers or complex electronic control circuits. The underlying theory and SPCF design are presented. In addition, widefield hyperspectral imaging using the SPCF is demonstrated for the first time on both model substrates and real-world samples.

Keywords: Biospectroscopy, Imaging, Instrumentation

Application Code: Bioanalytical

Methodology Code: UV/VIS
Previously, we demonstrated a side illuminated optical fiber for non-simultaneous measurement of multiple samples. In this paper, we adapted the previous set up with sources of non-overlapping spectral distributions to probe three samples simultaneously.

We used:

- A 20 cm long tapered optical fiber: diameter between 1 and 2 mm.
- Three LEDs of non-overlapping spectral distributions: peak wavelengths at 470, 570 and 645 nm.
- An LED driver to power the LEDs.
- Red, green and blue food dyes for calibration purposes: used to emulate commercially available colorimetric tests and an Ocean Optics USB-2000 spectrometer.

The procedure used was the following:

1. We prepared six calibrating solutions with the food dyes: concentrations between 0 to 5 drops/0.5 L of water.
2. The solutions were placed into cuvettes which were sandwiched between their corresponding LEDs and the optical fiber.
3. To maximize light absorption, the cuvettes with red, green and blue solutions were simultaneously illuminated with the blue, green and red LEDs, respectively.
4. The spectral distribution of the samples were obtained.

Light from each LED is absorbed by their respective samples, coupled into the fiber and transmitted to the spectrometer at the fiber tip. A total of six spectra, obtained from the different concentrations, were collected. Each spectrum has three different peak wavelengths which correspond to the three simultaneous measurements (see Figure). Accordingly, the intensity decreases with dye concentration. We plotted the calibration curves, fit them with an exponential function and obtained very high correlation coefficients: this confirms the data obeys Beer-Lambert’s Law. The confidence levels, CL, were 100% > CL > 99.9999%, 99.99% < CL < 99.99% and 99.99% > CL > 99.9% for the red, blue and green LEDs, respectively.

In conclusion, we demonstrated a rather inexpensive setup for simultaneous absorption measurements from multiple samples. We are now using this proof of concept for fluorescence, scattering and index of refraction measurements.

This work was supported by a Phase II grant from the SBIR Program of the US Department of Agriculture, Award No. 2011-33610-31155, National Program Leader William Goldner.


Conventional absorption spectrometers are designed with detectors whose dynamic range is inherently sufficient for the desired measurement, and typically observe only one or two diffraction orders. We describe an instrument with an inexpensive rectangular array CMOS detector using multiple orders to provide dynamic range that is the product of the dynamic range of the grating and of the camera. Such spectrometers require sophisticated software to generate a spectrum, but can be produced simply. They can observe spectra with a range of throughput/resolution tradeoffs simultaneously. We contrast the throughput, resolution, precision, and calibration of multi-order instruments with more conventional approaches, and critically evaluate what situations may benefit from this instrument geometry. The limitations of using consumer cameras optimized for snapshots and video recording as spectrometric detectors are also described, as are plausible means of overcoming the limitations. The spectrometer is mated with software to synchronize user actions, spectral measurements, and determination-specific data interpretation. Method examples will be presented, including visible reflectance and absorption. Human interfaces that hide the spectroscopy and chemometrics from the unskilled user, yet allow for “drill-down” to understand measurement complexity are also discussed.
The introduction of recent legislation, such as the EU Environmental Liabilities Directive 2004/35/EC, has encouraged the development of precise and robust analytical systems for identifying pollutants. However, the sheer number of toxic compounds which may require monitoring at any given time makes this a challenging prospect.

Quadrupole GC-MS methods often employ selective ion monitoring (SIM) for trace-level detection of target compounds. However, this protocol prevents retrospective searching of data for the latest contaminants of emerging concern. The use of time-of-flight mass spectrometry (TOF MS) can overcome this issue by providing highly sensitive detection whilst acquiring full range mass spectra, to allow both target and unknown identification in a single, rapid analysis.

This poster will illustrate the advantages of TOF MS by comparison with a modern quadrupole system operating in a regulated commercial environment. The reproducibility of quantitation and qualifier ion peak areas using this system will be shown, along with the ability to generate spectra that are a close match for those in the NIST library.
Applications of Gas Chromatography Mass Spectrometry

Introduction of an Improved Cyanopropylphenyl Stationary Phase with High Temperature Stability

For years, 1301-type stationary phases for capillary chromatography have provided an effective solution for the separation of many common volatile organic compounds. Although these cyanopropylphenyl polymers are well-suited for volatiles, they have historically suffered from poor thermal stability, resulting in higher bleed and low maximum operating temperatures. The poor thermal stability of these columns has also precluded their wide use in GCs using MS detection due to interference from bleed ions and source contamination from high bleed.

The new Rxi-1301Sil MS contains a more thermally-stable cyanopropylphenyl stationary phase that allows for low bleed at temperatures up to 320°C. This makes this column usable in MS instruments without the problems mentioned above. Additionally, the broader temperature range of this column allows for the analysis of less-volatile compounds that still benefit from cyano-type selectivity, such as the analysis of glycol ethers.

An evaluation of the performance parameters for the Rxi-1301Sil MS was performed. Evaluation criteria included MS bleed, selectivity, and lot-to-lot reproducibility. The Rxi-1301Sil MS exhibited less MS bleed than traditional 1301-type columns at a higher maximum temperature, while still exhibiting selectivity very close to that of traditional 1301 phases. Lot-to-lot reproducibility of several lots of columns was found to be excellent. This poster will present results of the column evaluation, as well as analysis of amyl alcohols in alcoholic beverages and MS analysis of glycol ethers.

Keywords: Beverage, Capillary GC, Gas Chromatography/Mass Spectrometry, Mass Spectrometry

Application Code: General Interest

Methodology Code: Gas Chromatography/Mass Spectrometry
Polymers and additives are used in a variety of subcutaneous and intravascular medical devices, such as sutures and drug delivery systems. These devices typically can be classified as being either absorbable or non-absorbable by the human body.

Natural materials, such as silk and catgut, are largely being replaced by synthetic materials. So, the reliability of these devices relies on the quality and proper application of these synthetic compounds. But regardless of their composition, the materials used are foreign to human tissue and will elicit a foreign body reaction to a greater or lesser degree.

This poster will demonstrate the analysis of several types of implanted medical devices from different manufacturers using pyrolysis-GCMS. Traditionally, pyrolysis has been used as a technique to identify polymers, which will be shown. But in addition, one or more "pre-pyrolysis" steps will be programmed first so that we can analyze for residual monomers, solvents, additives and contaminants, if present. These unwanted non-polymeric compounds or the incorrect quantity of an additive can often lead to product failure or worse.

Keywords: GC-MS, Medical, Polymers & Plastics, Pyrolysis
Application Code: Polymers and Plastics
Methodology Code: Gas Chromatography/Mass Spectrometry
Dinitrotoluene (DNT) is a common compound of interest for the instrumental detection of explosives. Several structural isomers of DNT are by-products or decomposition products of TNT while other isomers are commonly used as internal standards, particularly when performing gas chromatography / electron capture detection (GC-ECD) analysis of nitroaromatic explosives. Despite direct analytical confirmation that aliquots of commercially-obtained, purified solid DNT consist of only single DNT isomers (~99% purity), the headspace vapor above these solids has been observed to consistently contain multiple DNT isomers.

In this work, the headspace of purified DNT isomers, including 2,4-DNT, 2,6-DNT, and 2,3-DNT, were sampled by whole-air sampling via an online cryogenically-cooled injection system and solid phase micro-extraction (SPME). Samples were analyzed by gas chromatography / mass spectrometry (GC-MS) and compared to analyses of solutions made from the corresponding purified solid DNT isomers. Several sources of DNT were compared. This work demonstrated ratios of DNT isomers in the headspace that were inconsistent with those in the source material and were not attributable to impurities in the initial DNT stock or to differences in vapor pressure among different isomers. This evidence suggests a vapor-phase rearrangement as the source of the isomerization.

Keywords: Characterization, Gas Chromatography/Mass Spectrometry, Headspace, SPME
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography/Mass Spectrometry
Applications of Gas Chromatography Mass Spectrometry

An Improved Instrumental Method for Determination of Hydroperoxides in Jet and Diesel Fuels

The predominant mechanism by which liquid hydrocarbon fuels undergo degradation in storage or when thermally stressed, is free-radical autoxidation. Alkyl hydroperoxides are produced during autoxidation and can accumulate in the fuel. Thus the presence of hydroperoxides can serve as an indication that a fuel has undergone oxidation. Hydroperoxides have also been known to degrade fuel system elastomers and can serve as an oxygen source for further oxidative degradation. Traditionally, the standard test method for hydroperoxides in fuels is ASTM D3703, which is an indirect iodometric titration. This method requires up to 40 mL of fuel, is time consuming and is resource intensive. In addition, iodometry results in significant waste materials. Thus, an instrumental method for accurate determination of hydroperoxides in fuels was sought. An instrumental method has been published1, based on derivitization of hydroperoxides in aviation fuels with triphenylphosphene (TPP) and detection by gas chromatography with flame ionization (GC-FID) detection. The method measures hydroperoxides in fuels by quantifying the amount of triphenylphosphine oxide (TPPO) formed in reaction of TPP with measured hydroperoxides. However, it was found that this method was not applicable to hydroperoxides produced in diesel and marine gas oil fuels. We describe here how the TPP method was successfully modified and transitioned to an automated GC-MS-MS instrument, and extended to provide reliable measurements in a wider range of fuel types. The results of the automated GC-MS-MS method will be compared to the iodometric titration.


Keywords: Fuels\Energy\Petrochemical, GC-MS, Petroleum, Titration
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography/Mass Spectrometry
Improved Analysis of Petroleum Isomer Distribution Using Cold EI GC/MS

The hydrocarbon isomer distribution in petrochemicals contributes to many commercially important petrochemical characteristics such as boiling and melting points, octane number, combustion efficiency, flash point, viscosity, lubricity, solubility, and solvation power.

Electron Ionization Gas Chromatography / Mass Spectrometry (EI GC/MS) is a powerful and information-rich technique for qualitative characterization and quantitative analysis of the compounds in a petrochemical mixture. One of its most valuable functions is to provide the molecular weight of a compound. However, for high molecular weight or highly branched compounds this important ion may be small or absent because of energetic instability relative to its fragment ions. In that case analyte confirmation is more dependent upon measured retention time and comparison with established standards.

In contrast, Cold Electron Ionization GC/MS (Cold EI GC/MS) can improve petroleum isomer distribution analysis by substantially increasing the molecular ion peak intensity of a compound while retaining the EI fragmentation pattern for spectral library searching without modification to established GC methodologies. Enhanced molecular ion abundance allows plotting the molecular ion chromatogram for easy determination of isomer distribution, even for isomers with a high degree of branching and small or no molecular ion in conventional EI.

When used in a novel q-TOF configuration the enhanced molecular ion in Cold EI provides enhanced selectivity and valuable information on the isomeric content and distribution of petrochemical fluids.

**Keywords:** Biofuels, Fuels\Energy\Petrochemical, Gas Chromatography/Mass Spectrometry, Time of Flight MS

**Application Code:** Fuels, Energy and Petrochemical

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Applications of Gas Chromatography Mass Spectrometry

GC-MS Analysis of Essential Oil Extract from the Roots of Carpolobia Lutea - A Potential Tropical Pro-Fertility Plant

Several plants are used in folk medicine to treat infections that cause male infertility. Carpolobia lutea (G. Don) (Polygalaceae) is a plant used by practitioners of alternative medicine in South West Nigeria for the management of infertility gonorrhea, gingivitis, infertility, ulcer and malaria. This study is to extract and analyze essential oil from the roots of Carpolobia lutea. The fresh roots were air-dried, pulverized and the essential oil extracted into hexane by hydrodistillation over 4 hr period. The hexane in the extract was evaporated off and dried with anhydrous sodium sulphate. The essential oil obtained was analysed with GC model 7890 A and MS model 5975C of capillary column size 30 m x 250 µm x 0.25 µm film thickness which was packed with HP-5MS 5% Phenyl methyl siloxane. Helium was the carrier gas at a flow rate of 1 ml/min. The mass spectrometer (MS) was fitted with Chem.-Station software for processing the data. The temperature program of the column was initially 50[degree]C held at same temperature for 17 minutes, gradually increased to 200[degree]C at 40[degree] C/minute and finally increased to 240[degree]C at 15[degree]C/minute. Mass spectra were recorded using ionization energy of 70 eV. The molecular ions detected were identified by the MS library. Some of the compounds identified in the extract are dodecanoic acid, n-hexadecanoic acid, 13-octadecenal and oleic acid. The presence of fatty acids in the essential oil may be useful for the management of bacterial and fungal infections which may be implicated in male infertility factor. Thus the plant extract may be useful in the management of male infertility factor arising from bacterial/microbial infection.

Keywords: Analysis, Gas Chromatography/Mass Spectrometry, Natural Products
Application Code: Drug Discovery
Methodology Code: Gas Chromatography/Mass Spectrometry
The Volatile Organic Compounds (VOCs) are a class of organic chemicals having high vapor pressures at room temperature (low boiling points), which cause a large number of molecules to evaporate or sublime, into the gas phase.

In the recent years, the presence in Air of Toxic Organic Compounds such as pesticides, pharmaceuticals, explosives, chlorine disinfection byproducts, petroleum hydrocarbon and chlorinated solvents have been continuously increasing.

In accordance to EPA Compendium Method TO-17 for the determination of Volatile Organic Compounds in Ambient Air Using Active Sampling onto Sorbent Tubes, this work investigate the analytical performance of a Thermal Desorber coupled with a Gas Chromatograph/Time of Flight Mass Spectrometer System (TD-GC-TOF MS). The emissions are collected into a sorbent material filled tube. After sampling, the absorption tube is placed in the Thermal Desorber where it is heated. The Volatile Compounds are then desorbed by a flow of inert gas and transferred to a narrow-bore packed trap. This last one is filled with one or more sorbent material and is kept at a low temperature. Once the refocusing step is completed, the trap is instantaneously heated providing a rapid analyte transfer into the GC-TOF MS system.

Keywords: Analysis, Environmental/Air, Gas Chromatography/Mass Spectrometry, Time of Flight MS
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Applications of Gas Chromatography Mass Spectrometry

Effects of Pollution on the Religious Activity of Individuals Along the Ganges River: An Interdisciplinary Pilot Study with Sociological and Analytical Chemistry Based Approaches

The Ganges River (India) is a highly socially and economically significant river, being an important focus for religious activity as well as a main water source for the agricultural, industrial, and waste removal needs of 400 million people. Increasing utilization of the river’s water by these practices may potentially affect religious activities and customs that have existed for several millennia.

In this pilot study, we surveyed 150 individuals at six sites of religious significance along the length of the river, from December 2012 to February 2013, during the Maha Kumbh Mela. Water samples were concurrently taken and analyzed for polychlorinated biphenyls (PCBs), organochlorinated pesticides (OCPs), and polycyclic aromatic hydrocarbons (PAHs), using micro-electron capture or mass spectrometric methods. These data were spatially matched with our survey data that examined how and why religious activity and perception of the sacredness of Ganges River Water (GRW) was changing. Results suggest that while the religious entity of GRW is seen as pure and untaintable, the sacredness of GRW is reduced by a perception of high levels of visual and chemical pollutants. While previous research has demonstrated high levels of visual pollutants, we observed relatively low levels of the aforementioned chemical pollutants at sites surveyed.

Abstract Text

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Keywords: Elemental Mass Spec, Environmental/Water, GC-MS, Trace Analysis
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Applications of Gas Chromatography Mass Spectrometry

VOC and SVOC Emissions from Materials with Relevance to Vehicle Interior Air Quality: Regulation, Standard Methods and Analytical Implementation

Exposure to air pollutants has long been recognised as a cause of health problems. Historically, pollutants from vehicle exhaust and the burning of fuels were of primary concern, but as urban air quality has generally improved, attention has shifted to vehicle interior air quality (VIAQ). Emissions of volatile and semi-volatile organic compounds from car interiors can have an adverse effect on VIAQ, raising concerns for passenger health & safety. As a result of these concerns, VIAQ is of growing importance to the automotive industry, culminating in the development of harmonised methods to quantitate chemical release from materials used in cars. Methods generally specify the use of environmental chambers, vapour sampling onto sorbent tubes and analysis by thermal desorption (TD) with GC-MS. However, the broad range of sample types and the presence of target compounds at ultra-trace levels, often within complex matrices, provide a challenge to analytical chemists. Comprehensive 2D gas chromatography coupled with time-of-flight mass spectrometry (GCxGC-TOF MS) offers a potential solution by combining enhanced chromatographic separation with exceptional sensitivity. Select-eV ion-source technology extends this capability by enabling both hard and soft electron ionisation with no loss in sensitivity. The availability of full-fragmentation spectra for confident library matching is complemented by the ability of Select-eV to enhance diagnostic ions and distinguish between similar compounds. Fragment ions that ordinarily dominate high matrix samples become greatly reduced, enhancing selectivity and further improving detection levels. This holistic approach to material emissions profiling, combining TD-GCxGC-TOF MS with Select-eV ionisation technology, provides both enhanced separation and extended limits of detection for confident identification of a wide range of target compounds in a single analysis.

Keywords: Gas Chromatography/Mass Spectrometry, Polymers & Plastics, Time of Flight MS, Volatile Organic Co
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Dietary supplements are made from naturally derived ingredients (e.g. botanical) and may contain environmental xenobiotic contaminates. Persistent organic pollutants (POPs) in dietary supplements can be a source of xenobiotic toxins in the human body and cause detrimental impacts on human health. However, few methods exist for the extraction and quantitative determination of POPs in dietary supplements. Development of an efficient and precise method to extract and analyze POPs in dietary supplements is of significant importance to the regulation of dietary supplements.

In this research, a method was developed for organic toxins listed in EPA Method 625 and the Stockholm Convention. Samples were spiked with isotopically enriched analogues of the analytes into the preferred extraction solvent. Stir-bar Sorptive Extraction (SBSE) method optimization and Isotope Dilution were conducted and the optimized SBSE method was validated for gas chromatography–mass spectrometry (GC-MS) by analyzing blank-subtracted dietary supplements accurately spiked with EPA 625 analytes and enriched isotopic standards.

Desorption of the analytes was accomplished using thermal desorption–gas chromatography–mass spectrometry (TD-GC-MS) and quantification was made by isotope dilution mass spectrometry (IDMS). Following validation, the SBSE-TD-GC-MS method was used to analyze commercially available dietary supplements from U.S. and Chinese manufacturers. Specific patterns of organic contamination were found among the different manufacturers and varying points of botanical origin.

**Keywords:** Extraction, Food Contaminants, Gas Chromatography/Mass Spectrometry, Isotope Ratio MS

**Application Code:** Food Contaminants

**Methodology Code:** Gas Chromatography/Mass Spectrometry
DNA and cells of the human body are constantly exposed to attacks of oxidative environment. These attacks can be divided into two categories as exogenous and endogenous. Exogenous sources of oxidation relate to specific exposures of the organism to ionizing radiations. Endogenous attacks correspond to natural origin such as through cellular signaling and metabolic processes. During these attacks, although not in all cases, the primary damage is being induced by reactive oxygen species (ROS) and reactive nitrogen species (RNS). Two of the most common endogenous DNA base modifications are 8-oxo-7,8-dihydroguanine (8-oxoGua) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine. We investigated the levels of modified bases in calf thymus DNA by gas chromatography-mass-mass spectrometry (GC-MS/MS). Methodologies using GC-MS/MS have been developed to accurately measure the oxidation products of DNA with excellent sensitivity and selectivity in vitro. This work reports the effect of resveratrol, carvacrol and cyanidin-3-glucoside antioxidants on the endogenous levels of modified DNA bases in vitro. The levels of some modified bases increased, whereas those of others decreased. Measuring only 8-OH-Gua or 8-OH-dGuo would not have revealed contrasting changes in product levels by antioxidants. The results show that the types and yields of modified bases depended on the experimental conditions on the type of DNA damaging agent or oxidizing agents.

References
Gas chromatography tandem mass spectrometry has enjoyed resurgence especially in applied markets in recent years. A critical part of this technique is the development of robust SRM methods. This can be challenging due to the sheer number of precursor ion options for some compounds and at the same time, the lack of precursor ions for other compounds. In addition, the potential of matrix interferences corrupting SRM transitions requires that additional transitions be optimized. Choosing and optimizing SRM transitions is time-consuming and there is little written guidance.

This work summarizes the challenges and makes recommendations for choosing and optimizing SRM transitions specifically for pesticides. Transitions for over 200 pesticides were optimized and from that guidelines have been developed to help shortcut the process of SRM method development. Intensity and selectivity are weighed for choosing the best transitions and candidate transitions were vetting in food matrices.

Keywords: Analysis, Gas Chromatography/Mass Spectrometry, Method Development, Pesticides
Application Code: Food Safety
Methodology Code: Gas Chromatography/Mass Spectrometry
Applications of Gas Chromatography Mass Spectrometry

Volatile and Semi-Volatile Profile Comparison of Whole versus Dry Homogenized Wheat, Rye and Barley Grains by Direct Thermal Extraction GC/MS

Introduction:
The ability to profile volatile and semi-volatile components present in grains without the use of solvent extractions has several advantages including improving sample throughput, reducing the chance of a volatile component being “lost” in the extraction process and eliminating the need for solvent disposal. This study utilizes the advantages of direct thermal extraction GC/MS to profile whole grains of wheat, rye & barley as harvested and after being homogenized into a dry powder form. Direct Thermal Extraction GC/MS provides for fast analysis with no carryover problems that can be associated with other GC/MS techniques.

Method:
A small sample of each grain was analyzed by placing approximately 10 mg in a 4 mm id thermal desorption tube. The tube was then placed in a commercial thermal desorption system coupled to a GC/MS and desorbed. During the desorption process a cryotrap was cooled to -60C to trap and cryofocus the components. After desorption is complete the trap is ballistically heated to release the volatiles and start the GC/MS method. This process was repeated after the grains were dry homogenized to compare the volatile & semi-volatile profiles.

Preliminary Data:
A variety of small grains were analyzed by Direct Thermal Extraction GC/MS. The Direct Thermal Extraction GC/MS was able to detect the volatile and semi-volatile components of each sample. We were able to detect a complete profile of volatile and semi-volatile components from wheat, barley and rye grains both whole grains as well as in the dry homogenized state. The Direct Thermal Extraction GC/MS provided a quick way to evaluate the sample profiles, eliminating the need for solvent extractions.

Keywords: Agricultural, GC-MS, Thermal Desorption
Application Code: Food Science
Methodology Code: Gas Chromatography/Mass Spectrometry
Dang Gui root (Angelica sinensis), is widely used in traditional Chinese medicine to treat a wide range of ailments. Extracts of the root contain numerous constituents associated with the biological activity of this plant including several alkylphthalides (butylphthalide Z-ligustilide and Z-butylidenephthalide), and ferulic acid. Extraction, purification, and analysis of these constituents has been achieved using a wide variety of techniques including sonication or soxhlet extraction with aliphatic solvents followed by TLC or HPLC both as analysis and purification techniques.

As an alternative strategy, Dang Gui root pieces were first extracted using supercritical fluid extraction (SFE). Extracts were analyzed and components identified using Ultra Performance Convergence Chromatography (UPC2) combined with mass spectrometry. Identified components were then isolated and purified using preparative scale supercritical fluid chromatography (SFC). Following purification, identity and purity of the isolated components were confirmed using UPC2.
Capillary electrophoresis is a powerful separation technique that can achieve high efficiency separations of charged analytes. However, one limitation of capillary electrophoresis is the adsorption of cationic proteins and peptides onto the inner surface of the capillary wall, due to the negatively charged silanol groups on the bare fused silica capillaries. This can lead to poor separation efficiencies and band broadening. Gold nanoparticles have been known to exhibit unique interactions with biomolecules, which has led to an increased interest in these particles for pharmaceutical applications and separation science. Our group is attempting to investigate two different approaches for the separation of a number of opioid peptides using gold nanoparticles. Approach 1: Using a recently reported method for the preparation of a gold nanoparticle modified capillaries\cite{1}. These capillaries produced by treating the inner surface of fused silica capillaries with 3-triethoxysilylpropylamine, followed by the introduction of freshly prepared citrate stabilized gold nanoparticles (particle size $25\text{nm}$). Approach 2: We are also attempting to separate opioid peptides using the same citrate stabilized gold nanoparticles using capillaries treated with a layer of a positively charged poly(diallyldimethylammonium) chloride, that would adsorb the negativity charged gold nanoparticles\cite{2}. The electroosmotic flow, apparent electrophoretic mobilities, resolution and plate numbers for the opioid peptides under both approaches are compared to those obtained by conventional capillary zone electrophoresis on bare silica.

Reference:
(1) Hamer, M., Yone, A., Rezzano, I., Electrophoresis 2012, 33, 334-339

Support from NIH is gratefully acknowledged.

Keywords: Bioanalytical, Capillary Electrophoresis, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Capillary Electrophoresis
Recent reports demonstrate a large sensitivity increase in CZE-ESI-MS systems that employ etched capillaries, potentially due to changes in spray density. Ion distribution in electrospray generated from an electrokinetically driven sheath flow CZE-ESI-MS interface was mapped. The sheathing and separation buffers contained either a 25 \( \mu \text{g/mL} \) solution of dimethylated bradykinin or a 25 \( \mu \text{g/mL} \) solution of isotopically labeled dimethylated bradykinin (+6 Da deuterium), respectively or vice versa. The ratio of peptides during constant infusion was monitored while rastering the electrospray emitter across the MS inlet orifice, effectively highlighting the density of ions entering through the capillary as well as any spatial inhomogeneity in the spray. Ion distribution was also monitored by infusing the fluorescent dye Rhodamine 6G into the spray, exciting with a 532 nm green laser, and rastering in a similar manner as before. Mapping was completed using either a standard capillary (150 \( \mu \text{m} \) o.d.) or a capillary where the outer diameter had been etched with HF (60 \( \mu \text{m} \) o.d.). Our results may suggest a more focused beam of peptides present while using an etched capillary, leading to a greater number of ions entering the mass spectrometer, and hence a more sensitive measurement.

**Keywords:** Capillary Electrophoresis, Electrospray, Isotope Ratio MS, Mass Spectrometry

**Application Code:** Bioanalytical

**Methodology Code:** Capillary Electrophoresis
Bottom-Up Proteome Analysis of Escherichia Coli Using CZE-ESI-MS/MS with Capillary Reversed Phase Liquid Chromatography Prefractionation

Reversed phase liquid chromatography (RPLC)-electrospray ionization (ESI)-MS/MS is the usual analytical technique for bottom-up proteomics because of its good compatibility with ESI-MS/MS and high resolving power for peptide separation. However, the bias of RPLC-MS against small and hydrophilic peptides makes it essential to have a method with orthogonal separation mechanism providing complementary information. Capillary electrophoresis (CE) is a potential alternative. Capillary zone electrophoresis (CZE), which is the simplest model of CE, employs very simple instrumentation. With the development of an improved CZE-MS interface, CZE-MS/MS has recently attracted increasing attention for MS-based proteomics.

CZE has two primary advantages. First, it provides fast and efficient separation than RPLC. The flat flow profile of CZE leads to narrower peaks and better resolution. Second, the orthogonal separation mechanisms of CZE and RPLC could produce complementary information of the analytes. Nevertheless, the significant disadvantage of CZE is limited loading capacity, 1-3 orders of magnitude smaller than RPLC, which causes difficulty in identifying complex samples.

Sample prefractionation before CZE-MS/MS analysis can improve the peptide and protein identifications, so I developed a C18-capillary RPLC (cRPLC) based peptide fractionation method for CZE-MS/MS based bottom-up proteomics.

Around 50 µg E. coli peptides were loaded to the cRPLC column, and 61 fractions were obtained. Through the automated CZE-ESI-MS/MS system, 1,200 protein groups and 6,563 peptides were identified from the 50 µg E.coli digest. This result largely increased peptide and protein group IDs from the previous report, demonstrating the potentiality of CZE-MS in bottom-up proteomics. With the further improvement in CZE-ESI-MS/MS setup and prefractionation method, CZE-ESI-MS/MS may lead to better application in proteomics.

Keywords: Capillary Electrophoresis, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Micelles formed from bile salt aggregates are capable of resolving chiral isomers of binaphthyl compounds through micellar electrokinetic capillary chromatography (MEKC). Optimal resolution depends on a number of factors including the identity and concentration (10.0 to 100 mM) of the bile salt as well as the concentration of analyte enantiomer (0.10 to 3.00 mM). Here, the chiral isomers of model compounds such as 1,1'-binaphthyl-2,2'-diyl hydrogen phosphate (BNDHP) were separated with MEKC using various concentrations of the bile salts cholate (CA), deoxycholate (DC) or taurodeoxycholate (TDC). The best resolution and efficiency were observed with TDC-mediated MEKC, and solutions of TDC were analyzed with NMR to further investigate the TDC micelle. Relatively low bile salt concentrations (10.0 to 20.0 mM) and the lowest analyte concentrations (0.10 mM) provided the best MEKC resolution for all three bile salts. The critical micelle concentration (cmc) for each bile salt, demonstrated by the onset of chiral separation, differed across the three bile salts with the lowest cmc observed using TDC. TDC solutions were analyzed via 1D $^1$H NMR without analyte present, with R-BNDHP in solution and with S-BNDHP in solution at six different cholate concentrations (3.00 mM, 10.0 mM, 20.0 mM, 30.0 mM, 60.0 mM and 80.0 mM). Data from both MEKC and NMR experiments will be presented with the goal of developing an overall understanding of the factors that give rise to micelle formation and chiral separation.

Keywords: Capillary Electrophoresis, Chiral, Chiral Separations, NMR
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
A novel method based on microemulsionelectrokinetic chromatography (MEEKC) with reversed electrode polarity stacking mode (REPSM) was developed and validated for the simultaneous determination of dexamethasone sodium phosphate (DEX-SP) and the active drug dexamethasone (DEX) in rabbit plasma. The optimum separation was achieved when using a microemulsion background electrolyte consisting of 30 mM borate buffer (pH 9.2), 20 mM sodium dodecyl sulfate (SDS), 0.8% ethyl acetate and 2% 1-butanol. Electrophoretic separation was carried out at 24°C and at +23 KV. For the purpose of sample stacking, the following program was applied: +23 KV (0-0.6 min) then -23 KV (0.6-1.5 min) and finally +23 KV for the rest of the run. The analytes were base-line resolved within 12 min. Using hydrochlorothiazide (HCT) as internal standard, the peak area ratios were found to be linear in the concentration range (0.1-2.0 µg/ml, DEX-SP and 0.05-2.0 µg/ml, DEX). The applied stacking mode increased the detectability of the method by about 40-fold with limit of detection and quantitation of (0.03, 0.1 µg/ml, DEX-SP) and (0.02, 0.05 µg/ml, DEX). The developed method was fully validated according to the ICH guidelines. This optimized method was applied for the determination of DEX-SP and DEX in rabbit plasma samples previously treated with I.V. injection of DEX-SP. The in-vivo results thus obtained were used to study the pharmacokinetics of the cited drugs in rabbit plasma.
Capillary Electrophoresis (CZE) separates ions by their mass to charge ratio rather than hydrophobicity, which is more efficient for proteins. CZE also has an advantage over liquid chromatography in separation speed. This two features of CZE make CZE-ESI-MS/MS a high throughput screening method for quality control of monoclonal antibodies. Top-down analysis of intact antibody requires ultra-high resolution mass analyzer (i.e. FTICR) due to its high molecular weight (~150 000 Da). By reducing intact antibody, it breaks into light chains and heavy chains which are 50 000 Da and 25 000 Da, respectively, so more accessible mass analyzer such as Orbitrap can be applied for antibody analysis. In this work, capillary zone electrophoresis was coupled to LTQ Orbitrap Velos to analyze reduced monoclonal antibodies. Antibody was first denatured, reduced and alkylated. During desalting with ziptip, sample was eluted in 50% acetonitrile and 35% acetic acid in water to prevent it from precipitation. Sample can be preserved in solution longer than 1 month compare to dozens of minutes with only 50% acetonitrile. LPA-coated capillary was first directed infused with standard protein solution to further block the inner capillary wall. Total analysis time was 15 min with a separation window of ~5 min. Heavy chain and light chain were baseline separated for single monoclonal antibody. The mass of heavy chain and light chain were calculated by MagTran. For a mixture with two antibodies, light chains were baseline separated, while heavy chains were partially separated.

This work was funded by the National Institutes of Health (R01GM096767) and Eli Lilly Company through the Lilly Research Award Program.

Keywords: Bioanalytical, Biopharmaceutical, Capillary Electrophoresis, Proteomics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Capillary Electrophoresis
Aptamers are short, synthetic oligonucleotide sequences that can selectively bind to targets with high affinity. Selection of aptamers is accomplished by the Systematic Evolution of Ligands by EXponential enrichment (SELEX). The target is incubated with a random sequence oligonucleotide library and target-bound sequences are separated from the unbound pool to generate a higher affinity library for use in subsequent selection rounds. Capillary electrophoresis (CE) has been used for the selection of large protein targets and requires fewer rounds of selection than conventional SELEX methods. Unlike large targets, small molecule targets bound to DNA sequences show little to no change in electrophoretic mobility, resulting in poor separation from the unbound sequences. To solve this problem, quantum dots (QDs) with covalently bound target molecules can be employed to effectively increase the size of small molecular targets and allow efficient separation based on the QDs mobility. As proof of concept for the use of QDs in CE-SELEX we used commercially available QDs conjugated with streptavidin to show that QD-target-DNA complexes can be separated from unbound DNA sequences. Laser-induced fluorescence-CE and fluorescently labeled DNA allowed for real-time identification of DNA-target binding by fluorescence resonance energy transfer between the DNA fluorophore and the QDs, increasing the latter's fluorescent intensity. The dissociation constant of target-bound libraries was found to decrease throughout selection rounds until a minimum was reached.

This project was funded by Eastern Michigan University.
A capillary electrophoresis with laser induced fluorescence (CE-LIF) instrument was constructed with a low limit of detection. A calibration curve for this instrument was created using the product of the AttoPhos substrate system. Single enzyme molecules will be examined through CE-LIF to determine their individual activity. The calf intestinal alkaline phosphatase (ciAP) enzyme will be studied first in these experiments; a single enzyme molecule will be injected into a capillary filled with substrate, and several incubations will be performed at different points along the capillary. Each of these incubations with the AttoPhos substrate produced a pool of highly fluorogenic product known as AttoFluor, which will migrate through a highly sensitive laser-induced fluorescence detection system. The areas of the peaks produced will be used to create a kinetic plot to determine the activity of the enzyme with high precision. These experiments will be adapted for use with mass spectrometry and different enzymes in the future.
DNA damage is the underlying cause for mutations leading to cancer. It can be caused by a number of different agents ranging from chemical compounds to radiation sources. Detection of these different types of damages is important for understanding the mechanisms by which DNA damage is caused and propagated, particularly under real-world conditions of exposure to multiple sources. DNA damage from ultraviolet radiation (UVR) is caused by the formation reactive oxygen species and free radicals that result in the formation of two main damages; cyclobutane-pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4PPs). CPDs are generally the most common and most toxic form of DNA damage that results from UVR; however, 6-4PPs are more likely to cause serious damage and induce potentially lethal mutations. The objective of this work was to develop a method to simultaneously detect CPDs and 6-4PPs in genomic DNA irradiated with UVB or natural sunlight using using capillary electrophoresis with laser-induced fluorescence (CE-LIF). Human cells were first exposed to varying amounts of UVB or natural sunlight to induce DNA damage. Genomic DNA was extracted and incubated with anti-CPD and anti-6-4PP primary antibodies attached to secondary antibodies with a fluorescent quantum dot reporter that emitted either red or yellow fluorescence. CE was used to separate the unbound antibodies from those bound to the photoproducts, and LIF with appropriate optical filters was used to separate the fluorescent signals from each QD to individual photomultiplier tubes for simultaneous photoproduct detection. Using this strategy, photoproducts were detected from ~6 ng of DNA under a low UVB fluence of 65 J m⁻² for CPDs or 195 J m⁻² for 6-4PPs. This assay was also the first to demonstrate the detection of CPDs in human cells after only 15 minutes of irradiation under natural sunlight.
Improving an In-Capillary Assay for Creatinine

The determination of creatinine, a marker for renal failure, is a common clinical analysis. Here we explore the use of an electrophoretically mediated microanalysis (EMMA) assay for creatinine. The EMMA analysis requires low volumes of biofluids and generates minimal waste, but suffers from relatively low sensitivity. One way to improve EMMA sensitivity is to concentrate the assay product (aka Jaffe product) within the capillary. Here, discreet plugs of highly mobile hydroxide ions are used to affect dynamic stacking of the anionic product of this in-capillary assay. Creatinine reacts with anionic picrate forming the Jaffe product, a red ($\lambda_{\text{max}} = 485$ nm), highly mobile anion. The goal of this project is to use hydroxide, an even more mobile ion, as the leading anion to perform dynamic transient isotachophoretic (tITP) stacking of the in-line generated product. Discreet plugs of sodium hydroxide have been successfully employed with the EMMA assay to determine creatinine concentrations in both “elevated” (> 2.0 mM) and “normal” (as low as 0.05 mM) clinical ranges. Moreover, samples of creatinine in various matrices including 18 M$\text{m}$ water, 125 mM sodium chloride, and deproteinized artificial serum were analyzed to see how ionic strength and various ionic content affect the in-line assay. Using a 33.5 cm, 50 $\mu$m i.d. capillary, the Jaffe stacking system was systematically investigated using pH 9.0 background electrolyte (60.0 mM borate), and discreet hydrodynamic injections of (i) hydroxide (0.1 to 0.6 M for 50 to 400 mbar•s), (ii) 150mbar•s of picrate (47 mM, pH 12.3), and (iii) creatinine (0.05 to 10 mM in various matrixes). In general, stacking improved with hydroxide concentration and relatively long plug lengths (300 mbar•s). Importantly, the assay also showed good linearity over the clinical range of creatinine concentrations.

Keywords: Capillary Electrophoresis, Electrophoresis, Medical
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
We present a flexible and low-cost fraction collector for preparative capillary zone electrophoresis. We have demonstrated its application to the isolation of putative aptamers against thrombin and aim to further expand upon the capabilities of the instrument. We incorporate a novel two-dimensional CE interface into the instrument. This interface houses two capillaries separated by an incubation cell. This system will result in a fully automated two-dimensional CE instrument capable of generating high-affinity aptamers in a high-throughput fashion.

Keywords: Capillary Electrophoresis, Instrumentation, Sample Handling/Automation, Sample Preparation

Application Code: Bioanalytical

Methodology Code: Capillary Electrophoresis
Electrophoretically Mediated Microanalysis (EMMA) is applied to the measurement of creatinine through the Jaffe reaction to efficiently perform this clinically significant assay on the nano-liter scale. In addition, transient isotachophoresis (tITP) is used to stack the Jaffe product produced during the in-line reaction; the tITP step is initiated by a plug of highly mobile hydroxide ions. Simul, a computer simulation program, is used to visualize the dynamics of the overlap and stacking processes, allowing a detailed understanding of this complex tITP and EMMA-based reaction system. Through simulations, the dynamic stacking throughout the entire duration of the reaction can be "seen" and can give a deeper understanding of the overall reaction dynamics. Recent results pointing to optimal conditions for the analysis in Borate buffers of various concentration and pH in addition to altering the concentrations and lengths of the hydroxide plug will be presented. Ultimately the careful characterization of this system may lead to a more general understanding of tITP and EMMA dynamics to allow for prediction of optimal conditions for in-line electrophoretic analyses.
Neutral oligosaccharides have proven to be a difficult class of compounds to analyze by capillary zone electrophoresis (CZE) coupled to electrospray ionization-mass spectrometry (ESI-MS). Capillary electrochromatography (CEC), a hybrid of CZE and liquid chromatography, offers a solution by using electroosmosis to drive separations through a packed capillary, generating chromatographic separations of neutral analytes. Monoliths are an attractive alternative to traditional stationary phases, as they have high stability and permeability and are easily adaptable to current needs. We present results from a hydrophilic monolithic capillary column for CEC-ESI-MS of a neutral oligosaccharide mixture. Preliminary work with maltose oligomers indicates that the columns couple well with our sheath-flow interface but further column modifications are necessary. After system optimizations, we will explore a more complex sample: the nested fragments of the human high mannose 11mer.
The analysis of airborne aldehydes and ketones first involves collection of the analytes by passing air through a cartridge containing 2,4-dinitrophenylhydrazine (DNPH). As the air passes through the cartridge, the analytes react with the DNPH to form hydrazones which are immobilized on the cartridge. The cartridges are then eluted with solvent and the DNPH derivatives can be analyzed using HPLC with UV detection.

Using a robotic sampler with a special tray to hold DNPH cartridges, the entire process of desorbing the analytes and injection of the samples into the LC-UV system can be easily controlled. Automating the desorption of these cartridges can result in significant improvement in accuracy and reproducibility as well as reducing potential experimental errors by the operator. The intuitive software includes tools that allow the desorption of a cartridge to take place during the chromatographic separation of a previously injected sample to ensure maximum sample throughput.

In this report, the automation of the online desorption and analysis of airborne DNPH derivatives by the robotic autosampler is discussed. Examination and calibration for a variety of aldehyde- and ketone-DNPH derivatives is described. Finally, DNPH cartridges collected from representative matrices are desorbed and analyzed online and the resulting precision data are provided.

Keywords: Environmental/Air, HPLC, Laboratory Automation, Sample Handling/Automation
Application Code: Consumer Products
Methodology Code: Sampling and Sample Preparation
Sandalwood (Santalum album L.) is a desired wooden base note for many fragrances in perfume and other scented products including Japanese incense. Typically the tree is grown for 12 years before the total harvest of trunk, branch and root. There are many related species giving rise to counterfeiting and confusion among consumers. The greatest component in distilled oil is alpha santalol which is usually present in a concentration of approximately 50%. The alpha santalol is a weak odor compound with beta santalol being the stronger odor compound more associated with sandalwood oil.

The yield of the wood will change over time with a transition from beta to alpha santalol reported. There are more than 234 compounds that have been identified in sandalwood extract to add to the overall experience with ongoing research still identifying others. Not all compounds have an odor that contributes to the overall scent and it is possible to identify tree source by the ratio of compounds present. There is also a synthetic iso bornyl cyclohexanol that is available and present in commercial fragrances.

Three oil samples were obtained from a commercial aromatic oil source and analysed using Gas Chromatography Olfactometry (GCO) with simultaneous mass spectrometry detection, alongside samples of cedar wood oil and frankincense. The analysis showed the distinct differences in sandalwood oil from Indian and Western Australian sources and a synthetic sandlewood oil sample.

Keywords: Flavor/Essential Oil, Gas Chromatography, Gas Chromatography/Mass Spectrometry, GC
Application Code: Consumer Products
Methodology Code: Gas Chromatography/Mass Spectrometry
Silver engineered nanoparticles (AgNPs) are frequently used in consumer products from socks to washing machines, primarily due to their antimicrobial action. However, AgNPs are known to find their way into environmental waste streams and to have toxic effects on human health as well as aquatic life.

To better understand the impact of nanoparticles in the environment, several key characteristics need to be assessed, such as concentration, particle size, and size distribution. The recent development and advancement of single particle inductively coupled plasma mass spectrometry (SP-ICP-MS) provides very high sensitivity, the differentiation between dissolved and particles, particle size (if shape is known or assumed) and size distribution or agglomeration.

This presentation will discuss the application of SP-ICP-MS to a real world consumer product analysis. The hardware requirements that allow for very fast continuous data acquisition (100,000 points/sec at 10 us dwell time) as well as the commercially available software application which processes this large amount of data into usable particle analysis data will be discussed.

**Keywords:** Consumer Products, Nanotechnology, Quality Control

**Application Code:** Consumer Products

**Methodology Code:** Mass Spectrometry
Air fresheners are commonly used in households to mask unpleasant odors. Many types of fragrances are commercially available. The fragrance can be a complex mix of many components. The effectiveness of the freshener to mask the off odors over time can be directly related to the air concentration of the fragrance components. Therefore, an important aspect in product development is airborne concentration of the fragrance components.

This study describes the use of the GERSTEL GSS-FP sampler for the collection of fragrance compounds in air. The GSS-FP is a 28 position active sorbent tube gas/air sampling system designed to collect sample onto sorbent tubes. The sampler can collect multiple samples on a pre-programmed schedule or on demand from the user interface.

Air fresheners were dispersed in a room and samples collected using the GSS-FP. The sorbent tubes were analyzed by thermal desorption GC/MS. Several fragrance components contained in the dispersed products were monitored and quantified over time. The study also shows validation of an air sampling method for the compounds.

This type of experiment can be used to show the rate at which compounds decay in the room air as a function of time as well as identifying which compounds are important contributors to the perceived odor in the room.

Keywords: Air, Consumer Products, GC-MS
Application Code: Consumer Products
Methodology Code: Sampling and Sample Preparation
Niacinamide, a water soluble vitamin, is often used as a whitening agent in lotions. Niacinamide containing lotions have been shown to lighten the skin and give a more even complexion. Many of these lotions list niacinamide in the ingredients but do not give the concentration present. In this study, niacinamide was extracted from six different Olay products using liquid-liquid extraction. HPLC was then used to determine the concentration of the extracted niacin in each product. A spiked sample of an Olay lotion that did not contain niacin was used to determine the percent recovery for the extraction method. The percent recovery for the method was 99.22% with a percent relative standard deviation of 1.87%.

To test the method’s ruggedness, the experiment was performed by undergraduate students in a quantitative analysis course and also by students in an honors general chemistry course. The students’ results show that the experiment was simple to follow and the procedures were clearly written and gave consistently good results.
Enhanced LC-MS Sensitivity of Vitamin D Assay by Selection of Appropriate Mobile Phase

Subhra Bhattacharya
Thermo Fisher Scientific

We performed the LC-MS analysis of 25-OH vitamin D by using different mobile phase formulations. The mobile phases were a combination of additives such as volatile organic acids, low salt or the mobile phase without any additives. A small variation in pH and ionic strength introduced a substantial change in the SIM response of both 25-OH vitamin D2 and D3. Response comparisons were performed using both electro spray ionization and jet stream ionization mechanisms. Jet stream ionization showed significant increase in sensitivity compared to ESI when the mobile phase was used without any additive. Superior response was observed for low pH mobile phase in ESI. Trace amount of vitamin D could be detected by fine tuning a low pH mobile phase composition together with instrument operating parameters. To our knowledge, this is the first report of a comparative study of mobile phase effects on vitamin D sensitivity by LC-MS.

Chromatography, Liquid Chromatography, Liquid Chromatography/Mass Spectroscopy, Mass Spectro
Corrosion costs the petroleum industry an estimated $1.3 billion in non-productive time, materials and labor annually. Imidazolines prepared from fatty acids and amines are a widely-used class of chemical corrosion inhibitor, due to excellent performance and ease of handling. However, imidazolines actually comprise a mixture of several different chemical compounds, and the relative proportions of these species can have a large impact on both corrosion inhibition and product physical properties. The absence of gold standard analytical methods to characterize the active ingredients in imidazolines limit the understanding of the chemistry of these materials. It is for this reason that an SPE-LC-MS method was developed to supplement the chemical information afforded by bulk testing / wet chemistry methods (e.g. titrations etc). A high resolution time-of-flight (TOF) mass spectrometer was selected based on the fast scanning platform. This feature allows for low level sample interrogation in non-targeted analysis. Preliminary method development work was completed at the Biotage US Applications Lab (Charlotte, NC). The optimized sample preparation parameters were transferred to MWV (Charleston, SC). It is anticipated that this method will have significant impact in the formulation of new corrosion inhibitors for oil pipeline field applications as well as the quality control of finished products in manufacturing.
1-[2’-chloro-5’-sulphophenyl]-3-methyl -4-azo – [2”-carboxy-5”-sulphonic acid]-5-pyrazolone was prepared by diazotization of 4-sulphoanthranilic acid and then coupling with 1-[2’-Chloro-5’-Sulphophenyl]-3-Methyl-5-Pyrazolone in alkaline medium. It was characterized by elemental analysis and spectrophotometry studies. The transition metal chelates of the same were prepared with Cu2+, Ni2+ , Co2+ , Mn2+ and Fe3+ and characterized by Infra red spectral studies and physico chemical studies.

The PH of Ni(II)-[CSMACSP] was found 9.0. The molar absorptivity calculated was found 1.14x10^3 lit/mole.cm . The Beer’s law obey up to 14.26 ppm. and the ratio of M:L was found 1:2 using mole- ratio and Job’s method. The stability constant of the chelate were found 3.99x10^9 .The Gibb’s free energy was also calculated. The antimicrobial activity of ligand and its metal chelates were screened against various gram-positive (+) and gram-negative (-)organism. The results show that all these samples are more or less active agents against various organisms. The reagent were also studied % of Ni(II) in German silver. Thermal Analysis spectra and BOD/COD were also studied. DFT calculation was also done for its stability.
Herein, we report a new method for continuous detection of trace ions contained in pure water. The system consists of two mechanisms (a device that continuously removes either cations or anions selectively among the trace ions, and a virtually maintenance-free detector).

The container used for the ion removal device is made out of PEEK (PolyEtherEtherKetone). The container has three compartments that are isolated from each other by an ion exchange membrane. At both end of container, an electrode is installed.

Those three compartments are filled with either cation exchange resins or anion exchange resins. The continuous and selective removal of ions is accomplished by migration of trace ions from a central compartment toward either electrodes (cathode or anode) based on an electro kinetic phenomenon generated on surfaces of ion-exchange resins and membranes under the electrical field.

The measurement of trace ion amount is carried out with a contactless conductivity detector. The detector has a detection cell composed of PEEK tubing and three electrodes which are connected to the tubing. The cell has low void volume. Because electrodes have no contact with solutions passing through, an elution of metal ions due to electrolysis is prevented.

H+ and OH⁻concentration in a closed vessel of the pure water production process is calculated according to a change in electrical conductivity measurement of pure water upon removal of either cations or anions respectively.

The pH value of pure water is determined from an ion concentration difference between H+ and OH⁻.

As a detection limit of H+ and OH⁻with this proposed method is lower than 0.1 M, the pH value of solution, which is lower than 100 S/cm (mS/m), can be measured accurately.

Electrical conductivity detection of KCl solution that is pretreated with this removal device is shown in Figure 1 attached hereto.
Natural gas is a widely used source of energy; it’s a colorless, odorless, flammable gas and therefore odorized for safety reasons. The actual location where the gas is odorized is country depended. This can be done during production, at the country border or at different stages in the distribution network. Multiple, relative costly components can be used to odorize the natural gas. Preventing both ‘under’ and ‘over’ odorization there is a demand for a fast and accurate method to quantify odorant levels.

The natural gas suppliers association for an European-based country uses a Micro Gas Chromatograph for on-site analysis of both tetrahydrothiophene (THT) and tert-butyl mercaptan (TBM). This association is responsible for periodic odorant characterization and quantification for over 350 distribution points across the country’s entire natural gas network. Instead of taking a sample and bring it to the lab, which can take up to a few days before the result is known, the natural gas is directly analyzed using a Micro GC mounted in an off-road vehicle.

The Micro GC’s shoe-box size dimensions and low carrier gas consumption enables easy implementation in process applications and mobile laboratories. Direct, on-site analyses secures the integrity of the sample. Moreover, it leads to fast availability of the odorant’s concentration levels. In case of the Micro GC analysis, results are known within 90 to 120 seconds run time. Out-of-spec values can directly be communicated and corrective actions can be taken accordingly.

**Keywords:** Fuels\Energy\Petrochemical, Gas Chromatography, Portable Instruments, Process Monitoring

**Application Code:** Fuels, Energy and Petrochemical

**Methodology Code:** Portable Instruments
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**Abstract Text**

Process control is a major challenge for the chemical and petrochemical industries and in the field of energy. A better process monitoring ensures quality product, cost control, better maintaining of productivity and risk control. Direct analysis in the heart of the process is the most efficient way, but the market suffers from a lack of solution that could cover a wide range of applications. In this context, a collaboration between IDEEL and SRA Instruments companies was set up to develop solutions for on-line analysis answering to industrial needs.

A new generation of miniaturized gas chromatograph has been developed for the rapid analysis of liquid components. The analyzer is equipped with an on-line sampling valve developed and commercialized by SRA Instruments. This injection device uses a piston with a micro machined groove at its lower extremity, designed to contain the pressurized sample to be analyzed. The corresponding volume of liquid will be vaporized during the injection step thanks to the very fast and very powerful heating of the groove. Sample can be then injected directly in a short capillary column without saturation before detection by a µFID. This miniaturized flame ionization detector presents a strong interest for the micro analyzer, because of its low gas consumption, low power requirements for operation. It brings also similar sensitivity compared to conventional ones. Results obtained of all the technological building blocks are encouraging, this is the reason why a first prototype is presently being evaluated in IDEEL’s Laboratory of Industrial Analysis and tested on hydrocarbon mixtures.

Experimental evaluations are intended to measure performances of the instrument in terms of separation, repeatability and detection limit.

This new generation of micro-analyzer should extend the possibilities of in-situ process control. It represents a powerful tool to monitor complex liquid matrices.

**Keywords:** Fuels\Energy\Petrochemical, Gas Chromatography, On-line, Process Monitoring

**Application Code:** Process Analytical Chemistry

**Methodology Code:** Process Analytical Techniques
Honey is a valuable food commodity that gains a lot of attention due to its potential health benefits and usage to sweeten foods and beverages. To save on manufacturing costs, honey is susceptible to deliberate adulteration with sugar syrups such as corn syrup. Honey adulteration does not pose significant health problems, but it negatively influences market growth and consumer confidence. Fourier transform infrared spectroscopy (FTIR) with attenuated total reflectance (ATR) was used to collect absorption spectra of various commercial honey samples. Partial least-squares (PLS) was used to process FTIR spectra to determine the potential adulteration of the honey samples. The techniques presented in this work provide an alternative method to determine the purity and authenticity of foods, such as honey, and other natural products.

Keywords: Chemometrics, FTIR, Spectroscopy
Application Code: Food Safety
Methodology Code: Vibrational Spectroscopy
The verification of provenance is of vital importance in establishing food authenticity. For high value foods, the country of origin has a dramatic effect on the price, as such; falsifying the provenance of foods is an attractive proposition to fraudsters. A prime example is olive oil, being marked incorrectly from desirable countries or regions, such as North African Olive being identified as Italian or Spanish. Whilst various techniques such as trace metal analysis or isotopic ratios have been shown to separate different geographical classes, other factors such as year on year crop variations and changing weather conditions have caused these separations to be less conclusive. Using more than one technique and/or chemometric analysis to differentiate can be more conclusive. This work uses advanced analytics and visualization software with in built principle component analysis function or PCA to separate the geographical origin of olive oil utilizing GCMS data, Wine using ICPMS data and Whisky using ICPMS and LCMS data combined.

Keywords: Chemometrics, Food Safety, ICP-MS, Liquid Chromatography/Mass Spectroscopy

Application Code: Food Safety

Methodology Code: Data Analysis and Manipulation
A multi-residue method, based on programmed temperature vaporization gas chromatography triple quadrupole tandem mass spectrometry (PTV-GC-MS/MS), was developed for rapid determination of 54 kinds of pesticide residues in vegetables and fruits. We injected extracts of spinach, cucumber, apple, tomatoes, pepper, cabbage, cowpea, Chinese chives from the updated QuEChERS sample preparation method. Average recoveries were 70~130% for 54 of the pesticides in 3 different matrix. Also, detection limits were <5 μg/kg for all but a few pesticides, depending on the matrix. LODs were determined as 3.3 times S/N. LODs were in the range 2.0-10.0 μg/kg. The quantification of pesticide residues was performed by GC–MS equipped with a programmed temperature vaporizer (PTV) inlet, which can remarkably reduce the background noise and column burden by selective transfer of target analytes onto column. It was demonstrated to be a simple, sensible, reproducible and environment friendly method for the determination of pesticide residues in vegetables and fruits.

**Keywords:** Food Safety, Gas Chromatography/Mass Spectrometry, GC-MS, Pesticides

**Application Code:** Food Safety

**Methodology Code:** Gas Chromatography/Mass Spectrometry
A fast method was developed for the simultaneous analysis of 174 pesticides in grape using gas chromatography tandem mass spectrometry (GC-MS/MS) in multiple reaction monitoring (MRM) acquisition mode. The samples were extracted by acetonitrile, and separated with liquid-liquid partitioning from water in the sample by salting out with sodium acetate and magnesium sulfate (MgSO4). The supernatant liquid was purified by C18 and primary-secondary amine (PSA) to remove most of the fats and pigments in samples. The treated samples were then subjected to MRM analysis for 174 pesticides using GC-MS/MS, every compound has 2 MRM transitions (primary for quantification, secondary for qualification), totally 348 transitions in 15 minutes. The result showed that the linear relation between peak area and concentration of target were good from 5 ng/mL to 100 ng/mL (r2 >0.99). The limits of quantitation (LOQs) of all the pesticides studied were 0.1 µg/kg. The average recoveries were 70%~130% of target compounds and the relative standard deviations (n = 6) were below 10% in grape spiked levels at 10 µg/kg. The results show that this method can be used to simultaneous analysis of 174 pesticide residues in grape.

Keywords: Agricultural, Food Safety, Gas Chromatography/Mass Spectrometry
Application Code: Food Safety
Methodology Code: Gas Chromatography/Mass Spectrometry
Significant concern has arisen over the widespread adulteration of dietary supplements with synthetic phosphodiesterase type-5 (PDE-5) inhibitors sildenafil (Viagra®), tadalafil (Cialis®) and vardenafil (Levitra®) and their numerous structural analogs. Although mass spectrometry is being successfully employed by advanced investigators, regulatory agencies, and, increasingly, contract testing facilities, cheaper, and more accessible alternatives are desirable to enable mid-range QC laboratories to perform effective screening. Typical physiologically relevant dosages for the PDE-5 inhibitors start at single-digit milligram amounts; adulteration at lower amounts renders such remedies inefficient. However, presence of 50-100 mg offending substances is not uncommon. Dietary supplement matrices add additional degree of complexity to sample preparation, and limited solubility of these molecules in typical solvents could be problematic. Therefore, analysis of the solid matter without relying on elaborate sample preparation procedures, and complex analytical techniques, is desirable. The crystal morphology of the PDE-5 inhibitors may offer a rapid means of identifying their presence in dietary supplement matrices. Polarized Light Microscopy can be used to screen for the presence of PDE-5 inhibitor contamination, and FTIR Microscopy can then be used to further verify the identity of suspect crystalline inclusions, or at least, suggest their structural category. This combined microscopy approach is utilized to examine a number of dietary supplements purchased online and in US retail locations, to demonstrate feasibility of this methodology in erecting a barrier to adulteration, and permit rapid screening of potentially tainted dietary supplements.
Phthalate esters (PAEs) are a group of commercial chemicals widely used to make plastics more malleable and help lotions penetrate skin. A number of phthalate esters are known to cause birth defects or reproductive harm. As we all known, alcoholic drinks have always been popular around the world. In alcoholic drinks production, plastic containers are typically used in the storage and transportation process, which could make some phthalate esters leak easily from PVC tubes or vessels as well as plastic caps. The aim of this study is to determine the level of phthalate esters migration in alcoholic drinks by fast liquid chromatography-electrospray tandem mass spectrometry. This method is simple and rapid with acceptable sensitivity to meet the requirements for the analysis of PAEs in alcoholic drinks.
Wool Packing or No Wool Packing in a Splitless GC Inlet Liner—What is Better for Pesticide Analysis? A Case Study with a QuEChERS Strawberry Extract

Pesticide chemists usually avoid glass wool packing for splitless injection GC because, sometimes, lower responses are seen for active or thermally unstable pesticides (e.g., Carbaryl, DDT, Iprodione, etc.). But, properly deactivated quartz wool can not only be a valuable protector of the GC column from nonvolatile “dirt,” but also offers increased responses for pesticides because it stops them from hitting the bottom seal in a typical GC inlet, where they can be lost or degraded.

This study compared GC-MS response factors for a group of volatile to involatile pesticides from organochlorine, organonitrogen, organophosphorus, carbamate, and pyrethroids classes spiked into a QuEChERS strawberry extract, which was then splitless injected into single taper liners with wool and without wool. Not surprisingly to me, since I had seen it before with tobacco extracts (EPRW 2012), initial response factors were higher for the wool-packed inlet liner versus the liner with no wool. After 60 analyses of strawberry extract on each liner, some of the more difficult pesticides showed lower response factors on both liner types, but overall, the wool-packed liner still showed higher response factors versus the unpacked liner.

A final part of the study compared response factors for deactivated quartz wool liner packing to deactivated borosilicate wool liner packing. Deactivated quartz wool was significantly better for analyzing Dimethoate, Chlorothalonil, Carbaryl, Methiocarb, Dicofol, and Deltamethrin.

Keywords: Food Safety, Gas Chromatography, Pesticides, Sample Introduction
Application Code: Food Safety
Methodology Code: Gas Chromatography/Mass Spectrometry
The QuEChERS method was developed in 2003 by scientists at the USDA to speed up and simplify food sample preparations for multiclass, multiresidue pesticide analysis. Over the years, many modifications have been brought. This poster describes the use of the original extraction method and the AOAC approach for clean-up of 13 different pesticides, among those mostly used by Canadian apple producers.

The method involves 1) an initial extraction in an aqueous/acetonitrile system using magnesium sulfate (MgSO\textsubscript{4}) and sodium chloride (NaCl); followed by 2) a dispersive solid phase extraction (dSPE) as a clean-up step using primary-secondary amine (PSA), MgSO\textsubscript{4} and C18.

Target pesticides in the apple extract have then been detected and analysed by liquid chromatography coupled to electrospray ionization tandem mass spectrometer (LC-ESI-MS/MS).

This method has been validated in terms of recovery and reproducibility, and a limit of quantitation (LOQ) was also determined.

This work employed SiliCycle\textregistered Silia\textsuperscript{O} Quick extraction packets QE-0001-100P and SiliaQuick dSPE tubes QD-2006-15T.

Keywords: Contamination, Environmental Analysis, Isolation/Purification, Solid Phase Extraction
Application Code: Food Safety
Methodology Code: Sampling and Sample Preparation
An important aspect relating to the use of veterinary medicines in cattle is the presence of residues in milk. Antimicrobials constitute the largest class of compounds administered to livestock globally. This widespread use together with stringent food safety legislation necessitates the availability of rapid and sensitive analytical techniques for residue detection. Cost-effective, robust and broad-scope platforms, which can be easily implemented in routine control laboratories are of importance. Other considerations are the flexibility of analytical scope and the extent of compliance with internationally recognised validation criteria.

The workflow from screening to confirmatory analysis of antimicrobial residues, as required by European Union (EU) Regulation 2002/657/EC will be discussed. A simple dispersive solid phase extraction (d-SPE) procedure provided effective and simple sample clean up, where average recoveries were > 90 % for all multi class analytes.

Liquid chromatography separation, coupled with an accessible single quadrupole mass detector provided robust analysis and high sample throughout, where the screening target concentrations (STC) were less than the EU MRLs for each analyte. Suspect positive results identified by this screening method were directed for quantitative confirmatory analysis on a compact and high performance tandem quadrupole mass spectrometer.

The method was found to be fit for purpose, thus allowing for the qualitative and quantitative analysis of commonly administered antibiotics below EU regulatory limits in bovine milk.
Analysis of honey is important due to its nutritive and medicinal value. High concentration of essential metals and heavy metals can be toxic to both man and animals. Due to rapid increase in industrialisation in Kenya, there is corresponding increase in environmental pollution, hence increase in these metals in honey. Honey samples were collected from different geographical regions in Kenya. These included Nairobi, various parts of Rift Valley Province and Eastern Province. The selected Heavy metals (Pb, Cd, Zn) were determined by Flame Atomic Absorption Spectroscopy, while arsenic was determined by Hydride Generation-Atomic Absorption Spectroscopy. Essential elements (Na, K, Ca and Mg) were determined by Flame Atomic Emission Spectroscopy. The concentration of K, Na, Ca and Mg were found to range from 172.83+-0.02 to 781.52+-0.09 ppm, 98.04+-0.03 to 269.10 ppm, 19.33+-4.07 to 70.17+-3.90 ppm and 12.64+-0.43 to 41.88+-0.92 ppm respectively. The results obtained showed that most of the samples had a high concentration level of Zn with mean value of 0.19+-0.06 ppm followed by Pb with mean concentration of 0.16+-0.10 ppm and then Cu with mean concentration of 0.03+-0.01 ppm followed by Cd with mean value of 0.02+-0.01 ppm and finally As with mean value of 0.01+-0.01 ppm. The mean concentration level of Pb was found to be higher than 0.1ppm which was above standards set by the World Health Organization (WHO) and Kenya Bureau of Standards (KEBS) for food products.
Most continuous flow analyzers are designed to perform either Segmented Flow Analysis (SFA) or Flow Injection Analysis (FIA). Neither technique addresses the full range of reaction chemistries and analyses laboratories are called on to perform. Segmented flow analyzers require complex air injection and wetted solutions, while flow injection analyzers are less effective with chemistries requiring long reaction times. This poster will report results of a performance study on a new continuous flow analyzer that combines SFA and FIA techniques on a single instrument platform. Design and performance characteristics such as reduced detector noise and expanded calibration range of the new automated chemistry analyzer will be presented.
Generally, inductively coupled plasma - Optical emission spectroscopy (ICP-OES) is used to analyze gasification slags. However, tedious sample preparation process and availability of small amount of slags sample is of great concern. In order to overcome these problems, we propose the use of Laser induced breakdown spectroscopy (LIBS) for analysis of small quantities of slag samples. It is advantageous to use LIBS because it permits a rapid in situ sample analysis with little or no sample preparation. In this work, synthetic slags with chemistry falling within coal-petcoke mixed feedstock slags were prepared for the investigation. A small amount of synthetic slag sample placed on a double sided adhesive tape was used in LIBS experiments. Optimization of energy, gate width, and gate delay for well resolved spectra with good SNR and SBR was carried out. Careful selections of spectral lines of Al, Ca, Fe, Si, V which do not suffer from potential self-absorption were considered for LIBS analysis. Intensity ratios to reduce shot to shot fluctuations were used to develop calibration curves and result was compared to those obtained using ICP-OES. Furthermore, multivariate statistics and data analysis methods were used to ascertain the results.

**Abstract Text**

Generally, inductively coupled plasma - Optical emission spectroscopy (ICP-OES) is used to analyze gasification slags. However, tedious sample preparation process and availability of small amount of slags sample is of great concern. In order to overcome these problems, we propose the use of Laser induced breakdown spectroscopy (LIBS) for analysis of small quantities of slag samples. It is advantageous to use LIBS because it permits a rapid in situ sample analysis with little or no sample preparation. In this work, synthetic slags with chemistry falling within coal-petcoke mixed feedstock slags were prepared for the investigation. A small amount of synthetic slag sample placed on a double sided adhesive tape was used in LIBS experiments. Optimization of energy, gate width, and gate delay for well resolved spectra with good SNR and SBR was carried out. Careful selections of spectral lines of Al, Ca, Fe, Si, V which do not suffer from potential self-absorption were considered for LIBS analysis. Intensity ratios to reduce shot to shot fluctuations were used to develop calibration curves and result was compared to those obtained using ICP-OES. Furthermore, multivariate statistics and data analysis methods were used to ascertain the results.

**Keywords:** Atomic Emission Spectroscopy, Chemometrics, Elemental Analysis, ICP

**Application Code:** High-Throughput Chemical Analysis

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
In order to gauge the environmental effects of titanium dioxide, an abundantly produced nanomaterial, it is important to characterize its binding affinity to biological substances in aquatic ecosystems. Estradiol and ethinyl estradiol are of interest as these chemicals are common endocrine disruptors. The subsequent health defects associated with endocrine disruption are further complicated by estrogen-titanium dioxide complexes. To understand the extent of these effects, it is crucial to determine the binding constants of titanium dioxide to estrogens in different mediums. Bound estradiol is quantified using pH-mediated stacking in capillary electrophoresis. This method concentrates the analyte of interest, requiring microliter sample volumes and improving the limit of detection to the nanomolar range. Furthermore, the technique utilizes a mixture of cyclodextrins and lauryl sulfate (SDS) micelles to allow the partitioning and separation of neutral steroid hormones.

Initial studies involve the binding affinity of titanium dioxide to estradiol in a pure water medium, followed by the more complex matrices of albumin and aquatic biome mediums. Results of albumin-facilitated binding give insight into the effect of protein adsorption on nanoparticle-small molecule complex formation. The result of the binding study in aquatic biome enhances understanding of contaminant binding in river water and wastewater. This study also allows a better understanding of the toxicity of titanium dioxide with regards to reproduction and circulating steroid hormone levels in the blood.

Keywords: Bioanalytical, Capillary Electrophoresis, Quantitative, Separation Sciences
Application Code: High-Throughput Chemical Analysis
Methodology Code: Capillary Electrophoresis
High-Throughput Chemical Analysis

A Rugged C18 Stationary Phase for Accelerated Analysis

Fast analysis used to be limited to chromatographers with access to expensive UPHLC systems. The re-introduction of superficially porous particle (SPP) liquid chromatography columns to the marketplace has given everyone access to the increased efficiency and peak capacity to decrease their analysis times. The high amount of hydrophobic retention of a traditional C18 phase is well established and every chromatographer includes one in their cache. This new SPP C18 is an endcapped Octadecylsilane phase for fast, rugged, consistent, and reliable reverse phase chromatography. The particles provides higher efficiency for faster analysis and increased sample throughput on typical LC systems. With them we achieve UHPLC like speed and performance with the 2.7µm SPP and higher efficiency per unit backpressure than traditional fully porous silica supports with the 5µm SPP. The versatility of these columns makes them ideal for separations in bioanalytical, CMC testing, food safety, environmental, and other testing areas. Example separations on these particles will be shown to illustrate their contributions to efficiency and overall sample throughput.

Keywords: HPLC, HPLC Columns, Liquid Chromatography, Liquid Chromatography/Mass Spectroscopy

Application Code: High-Throughput Chemical Analysis

Methodology Code: Liquid Chromatography
In this study, a rapid method is described for detecting L-Ascorbic acid (Vitamin C, L-Ascorbate) concentrations from milk powder and other samples in the dairy industry. This method is adapted for use with an automated discrete analyzer which is capable of simultaneously analyzing multiple parameters using enzymatic, colorimetric or electrochemical (ECM) method principles. The automated L-Ascorbic acid method is based on the capability of tetrazolium salt MTT [3-(4,5-dimethylthiazolyl-2)-2,5 diphenyltetrazolium bromide] to reduce L-Ascorbic acid and other substances by means of the electron carrier PMS (phenazinium methosulfate) to form colored formazan.

For determination of L-Ascorbate, the assay was specifically designed to use a sample blank, where L-Ascorbate is oxidatively removed by Ascorbate Oxidase. Ascorbate Oxidase was available in a ready-to-use liquid format and the process to add the reagent was fully automated. The difference in absorbance at 575 nm between the sample and the sample blank was equivalent to the amount of L-Ascorbate in the sample. All results were calculated automatically. Samples requiring pretreatment were dissolved in 1.5% metaphosphoric acid (pH 3.5-4). Turbid samples were filtered prior to analysis.

This test can be used for a sample concentration range from 50-2500 mg/L. The method is very fast, requiring approximately 75 minutes for analysis of 100 samples without the need for any additional hands-on time. In method correlation studies, the same samples were measured by both HPLC analysis and the automated discrete analyzer method. Very good correlation was shown in the results.

Keywords: Analysis, Automation, Beverage, Food Science
Application Code: High-Throughput Chemical Analysis
Methodology Code: Chemical Methods
A new Ultra-fast Raman Chemical Analyzer, AcuScan2000 has been developed for molecules identification and quantification of organic as well as inorganic substances. Since Raman scattering produces molecules fingerprint, it is an idea universal detector for polarizable molecules that produce symmetry vibration as well as rocking and scissoring stretching. The Raman molecules detection specificity allows simultaneous identification and quantification of chemicals eluted from HPLC column. This report will describe the AcuScan2000 on-line HPLC-Raman instrumentation as well as applications of the AcuScan2000 system for the identification and quantification of HPLC column eluents such as pesticides and pesticide residues, food additives, drugs, etc.

Keywords: High Throughput Chemical Analysis, HPLC Detection, Raman, Vibrational Spectroscopy

Methodology Code: Vibrational Spectroscopy
The development and application of new ion chromatographic columns packed with ion exchangers of smaller particle sizes (e.g., 4 µm or smaller) have gained increasing interests in recent years since these columns bring out new opportunities to perform fast and high-resolution separations of ionic analytes. The new smaller-particle-size columns typically yield pressures higher than 3000 psi, which is the maximum operating pressure of the current generation of the Reagent-Free Ion Chromatography (RFIC) systems using electrolytic eluent generators. Therefore, there is a need to develop new RFIC systems and electrolytic eluent generators capable of operating at higher pressures.

In this presentation, we will report our recent efforts in developing new generation of electrolytic eluent generation devices for producing high-purity potassium carbonate and bicarbonate eluents using only deionized water as the carrier stream at pressures up to 5000 psi. We will describe the principle and operation of the new electrolytic eluent generators. We will demonstrate the advantages of using the high-pressure RFIC systems to achieve fast and high resolution ion chromatographic separations of target analytes in different samples.
Superficially porous particles (SPP) offer improved efficiency and performance over similarly sized traditional totally porous particles, with similar performance to sub-2um materials but without many of the drawbacks encountered with those materials. This is primarily due to a shorter mass transfer distance and substantially narrower particle size distribution of the particles in the column. Higher efficiency leads to improved resolution and possible time savings with SPP, hence their growing popularity for HPLC analyses. Columns using superficially porous particles are currently available in a wide variety of particle sizes, pore sizes and stationary phase chemistries to meet most analysts’ needs.

Often methods using totally porous particle columns can be directly transferred to SPP columns without adjustment. This is particularly true when similar or identical bonding chemistries are available on both types of particles. Method scalability is also facilitated by the increase in choices of particle sizes. Together, these qualities allow for quick and reliable method scaling or transfer, ranging from method development for preparative laboratories to methods for high throughput analyses, while also catering to the use of a wide range of instruments and pressure limitations. For those analysts looking to speed up analyses, SPP columns can offer shorter analysis times, and improved sample throughput. However, analysts must pay attention to the instrument configuration and connections for maximum performance. This work will address method transfer and scalability from totally porous particles, as well as among different varieties of SPP, and overcoming some common barriers of method transfer.

Keywords: HPLC Columns, Liquid Chromatography, Method Development
Application Code: High-Throughput Chemical Analysis
Methodology Code: Liquid Chromatography
Achieving Faster GPC Separations Using Novel High Pore Volume Columns and Conventional Instruments

GPC is an important technique for determining the molecular weight distribution of a polymer and for comparing batch-to-batch polymer quality. In common with other liquid chromatographers, GPC users are making more demands on the technique both in terms of speed of analysis and quality of results. These additional demands have to be balanced with the need to control costs, and the purchase of new specialized instrumentation to achieve performance gains may not be justified. The combined requirements for faster size-based separations and the use of conventional instrumentation could be achieved through the use of novel GPC column technology.

Columns packed with particles that have a very high pore volume deliver significantly increased resolution compared to a conventional GPC/SEC column set – this allows run times to be decreased, through the use of shorter column lengths and higher linear velocities, without sacrificing separation quality. Using industry standard, highly cross linked polystyrene/divinylbenzene (PS/DVB) particles with a diameter >/=3 µm maintains compatibility with conventional GPC instruments.

In this work, the results for the analysis of polymer samples run on an Agilent 1260 Infinity GPC/SEC system and conventional column set are compared to results obtained from analysis on the same instrument using columns packed with high pore volume particles. The comparison demonstrates that significant reductions in analysis time can be achieved while maintaining acceptable results quality, without the need for specialized instrumentation.

Keywords: Chromatography, High Throughput Chemical Analysis, HPLC, Polymers & Plastics
Application Code: Polymers and Plastics
Methodology Code: Liquid Chromatography
Abstract Text
Temperature affects chromatography. This fact is well known and this is why every good chromatographer will implement some form of temperature control in his/her experiments. With UHPLC, operating temperatures have risen to values very close to or even above the boiling points of typical solvents. Consequently, column ovens have evolved from simple units with fixed temperatures, to high-tech devices capable of heating up a mobile phase to the actual column temperature prior to entering the column, as well as the ability to cool mobile phases prior to entering the detector. In addition, viscous heating is a much discussed topic in UHPLC. When pressures increase to values above 400 bar the friction between the column and the mobile phase can generate additional temperature increases inside the column. Adiabatic heating is typically applied to remedy this situation. However, although it will generate a more uniform temperature increase across the column, the adiabatic approach does not prevent viscous heating. In this work we critically evaluate the various elements of the column oven to accurately determine the effects of different setting on chromatographic performance. Furthermore, we measured the degree of viscous heating that takes place inside the column and discuss how adjustment of chromatographic parameters can be used to maintain or even improve separation.

Keywords: Detection, Liquid Chromatography, Temperature
Evaluating the efficacy of psychotropic pharmaceuticals often involves in-vivo animal experiments with repeated sampling of brain extracellular fluids for their neurochemical content. By using a microdialysis probe, any changes in levels of biogenic amines and major metabolites can be monitored over time. This offers insights into the psychoactive nature of the drug candidate. Therefore, experimental samples must be repeatedly and accurately analyzed so that any possible changes of major neuroactive chemicals can be observed with reasonable temporal resolution. Rapid analytical techniques such as UHPLC with sensitive electrochemical detection provide advantages over traditional HPLC assays since the sampling rates can be increased due to shorter run times involved with analysis.

The analysis of biogenic amines and major metabolites in these samples is challenging since these compounds are labile and their levels in extracellular fluids from various brain regions are quite low due to active reuptake systems and catabolism. Transport of these compounds by diffusion processes across the microdialysis probe is limited, so only partial recovery of these chemicals from the extracellular fluid actually occurs. These issues present significant assay sensitivity challenges. Combining low microliter sample volumes with short collection periods means the assay must be capable of detecting picomolar levels of analytes. By using a specialized LCEC system, capable of handling the elevated pressures related to UHPLC operation while maintaining the full sensitivity requirements of the electrochemical detection to femtogram levels of detection, assay requirements can be fully satisfied. This work describes the analytical conditions for the successful measurement of biogenic amines and major metabolites in microdialysis samples.

Keywords: Bioanalytical, Electrochemistry, Liquid Chromatography, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Liquid Chromatography
Sample preparation is one of the most important processes to achieve a good analytical separation. In order to develop more appropriate sample preparation techniques, simplification, automation and miniaturization have been studied widely in the field of analytical chemistry, along with the development of alternative methods.

In this study, an online sample preparation/separation system for aqueous sample was developed with polymer-coated fiber-packed capillary. The system has been developed using an extraction capillary and two trap capillaries packed with a bundle of polymer-coated filaments. As a coating polymer onto the surface of the filaments, a silicone polymer, which is a conventional liquid stationary phase for capillary columns in gas chromatography (GC) was employed. Packed longitudinally with a bundle of polymer-coated filaments into a stainless-steel capillary (0.8 mm i.d., 150 mm length), the polymer-coated fiber-packed extraction capillary was prepared. Aqueous sample solution was pumped through the extraction capillary and the solutes were extracted therein. Taking advantage of the heat-resistant property of the polymer-coated fiber, the extracted analytes were sequentially eluted and roughly separated in the extraction capillary with a temperature-programmed run. Introducing two trap capillaries packed with a bundle of polymer-coated filaments (0.5 mm i.d., 100 mm length) as the interface, the developed extraction system was on-line coupled to micro column LC. The system allowed highly selective and sensitive analysis of benzoates in aqueous solution.

Keywords: HPLC, Temperature
Application Code: Environmental
Methodology Code: Liquid Chromatography
Multi-angle light scattering (MALS) detection has become an indispensable tool for polymer characterization. The coupling of MALS with high-performance size exclusion chromatography has provided a unique and attractive technique for obtaining absolute molecular weight information and molecular size information about macromolecular systems including both natural and synthetic materials. We have successfully developed new size exclusion polymer packing material for a column suitable for MALS analysis. Our new column allows for detection of polymers with a molecular weight range of approximately 10,000-100,000 daltons. This molecular weight range was particularly difficult for estimation by MALS due to interference from noise due to the packing material. Thus, our new column with low noise will allow MALS detection for a greater number of compounds with lower molecular weight.

We will introduce the feature of reduced noise as well as several basic features using MALS detection on our newly developed column with several applications.

Keywords: Light Scattering, Liquid Chromatography, Materials Characterization, Materials Science
Application Code: Polymers and Plastics
Methodology Code: Liquid Chromatography
Although levamisole is sometimes used as an anthelmitic therapeutic treatment for horses, there has been some concern that because it metabolizes to aminorex, an amphetamine-like drug, it has the potential for abuse, and so its therapeutic use has been banned in some countries. Moreover, it is thought to play a role in the in-vivo production of a significant performance enhancing drug, pemoline. Levamisole is also used as an adulterant in cocaine, its addition likely to extend the effect of cocaine by metabolising to aminorex just as the effect of cocaine diminishes.

Chiral analysis may play a significant role in understanding whether detected levamisole and/or aminorex are due to legal administration of drug products or illicit use for performance enhancement. The project employed several cyclodextrin-based chiral stationary phases and covers the method development and optimization processes. The resulting method may be used to differentiate enantiomers of tetramisole (levamisole and dexamisole) for drug product analysis and performance-enhancing activities in horse-racing. In addition, the method has been shown to provide separation of aminorex and cocaine from the tetramisole enantiomers and thus may be useful in cocaine related studies.
An ultraviolet (UV) absorbance detector for HPLC has been widely used in many fields. However, the UV detector is hardly used to detect sugar because of its almost non-existent UV absorption. Also, the UV detector is frequently used to detect peptides, but its sensitivity is low because the peptide bond has weak UV absorption at around 210 nm. It is well-known that most organic substances have strong light absorption in the far-ultraviolet (FUV) region below 190 nm. Therefore, if the detectable wavelength of the absorbance detector could be extended to a wavelength region below 190 nm, it could detect not only sugar but also peptides with high sensitivity.

In this study, we modified the UV detector for the wavelength region down to 175 nm for sugar and peptide analysis. The results reveal that the absorption peak of sugar lies at around 178 nm. The detection limit (S/N = 3) of fructose and glucose were 50 and 177 pmol, respectively (Fig.1). Also, the results clearly show that the peptide bond has a strong absorption peak at around 190 nm. The peptides (Met-Enkephalin and Gly-Gly) were separated by using a gradient elution method with water/acetonitrile at 190 nm and showed a detection limit (S/N = 3) of 2.4 and 2.7 pmol, respectively, which were five times larger than those at 210 nm. In addition, significant changes in the baseline due to the gradient elution were corrected with a newly developed gradient monitor based on near-infrared absorptiometry (Fig.2).

Keywords: Array Detectors, HPLC Detection, Peptides, UV-VIS Absorbance/Luminescence
Application Code: Quality/QA/QC
Methodology Code: Liquid Chromatography

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Herein, we report a new Non-Pneumatic (without vacuum) Membrane Gas Removal System that selectively removes CO₂ (H₂CO₃) gas dissolved in a solution. The system consists of two mechanisms (Cation removal mechanism and CO₂ removal mechanism).

Cation removal mechanism, which is often referred to as an ion suppressor in Ion Chromatography for anion analysis, removes cationic ions from the solution and converts HCO₃⁻ (CO₃²⁻) dissolved in the solution to CO₂. CO₂ converted from HCO₃⁻ (CO₃²⁻) is then removed from the solution by CO₂ removal mechanism.

CO₂ removal mechanism consists of an airtight seal vessel that encloses a gas permeable tubular membrane module and a disposal packet of CO₂ adsorber. In this mechanism, the dissolved CO₂ (H₂CO₃) starts to migrate out of the solution in accordance with a concentration diffusion theory based on a difference of CO₂ concentration between inside and outside of the tubular membrane. The temperature in the vessel is controlled at 37°C. The pressure in the vessel is controlled at 100 kPa.

The gas permeable tubular membrane is 4m long Teflon AF2400 (U-VIX Corp., Tokyo, Japan). The CO₂ adsorber is a tea bag like packet of commercially available polypropylene-nonwoven fabrics and is packed with very well dried OH type AE resin (Amberlite IRA402BL, Dow Chemical).

The vessel has two ports which are used to reduce pressure in the vessel.

The CO₂ concentration contained in the solution was decreased down to a level similar to the one of pure water by the system. The result is shown on Figure 1 attached hereto.
The increasing need for renewable fuel sources has led to the investigation of different methods that can maximize the bioethanol production from the fermentation of carbohydrate-rich biomass. Two most commonly used pretreatment methods for carbohydrate-rich biomass are enzymatic hydrolysis and mineral acid hydrolysis. However, enzymatic hydrolysis is a slow process and mineral acid hydrolysis requires neutralization and generates a waste stream. In this study, high temperature water and carbonic acid were used as an alternative to the conventional dilute mineral acid hydrolysis. Unlike mineral acids, carbonic acid generated from dissolved CO2 does not require post-neutralization and reduces the waste stream. A high pressure continuous flow reactor with pressurized carbon dioxide was employed for the hydrolysis of wet microalgae and other biomass. The reaction conditions such as pressure, temperature and residence time were optimized by monitoring the formation of simple sugars and degradation byproducts with HPLC. The yield of simple sugars from the direct treatment of whole microalgal biomass for 5 minutes at 210°C using 7 MPa CO2 was very low, but increased to levels similar to the dilute mineral acid hydrolysis when a small amount (0.05%) of sulfuric acid was added. The amount of thermal degradation byproducts such as 5-HMF and furfural was approximately one order higher, however, no inhibition was observed during the subsequent fermentation of hydrolysis substrates to ethanol.
Gold-coated iron oxide core-shell nanoparticles (IO-Au NPs) are of interest due to their potential uses in numerous biomedical applications including capture and detection of circulating tumor cells for early cancer diagnosis, enhancement of MRI and near-infrared photothermal therapies. In order for a nanoparticle to exhibit optical properties that are advantageous for a specific application, there are several significant components including shell thickness and particle size, but the largest contributing factor is the shape of the nanoparticle. Core-shell IO-Au-NPs have been synthesized in various shapes including, spheres, pins and stars, however, the mechanisms determining the final shape of the nanoparticles are not well understood. A systematic study of nanoparticle growth through the use of Transmission Electron Microscopy (TEM) will assist with future synthesis of nanoparticles tailored for specific methods. For this investigation, we have developed a TEM method for time-resolution of Au-shell formation and growth processes to form core-shell IO-Au NPs. Information regarding the Au-shell growth process may allow for the improvement of current synthesis methods to form more monodisperse anisotropic IO-Au NP samples with desired optical-plasmonic properties.
Microscopy Techniques for Nanotechnology and Biomedical Applications

Giant Lipobeads: Preparation, Characterization by Confocal Microscopy, and Mechanisms of Controlled Release

The hydrogel-liposome bi-compartmental structures (lipobeads or lipogels) combine biocompatible surface properties of lipid bilayer with the polymer network’s mechanical stability, loading capacity, and ability to release drugs controllably. Giant lipobeads (GLB, 3-500 [micro]m) are convenient objects for direct observation by optical microscopy and modeling their properties as drug delivery systems. We have prepared GLBs using two methods: polymerization within liposomal reactor and microgel-liposome mixing. In the first method, hydrogel forming solution was incorporated inside giant unilamellar vesicles (GUV) and exposed to UV light to provide polymerization within the GUV microreactors. In the second method, microgel beads prepared by means of inverse suspension polymerization were mixed with liposomes prepared by means of extrusion. The experimental steps, optimal parameters and conditions were justified for both methods. To distinguish between the lipid bilayer envelope and the hydrogel core using fluorescence and confocal laser scanning microscopy, red image of a phospholipid layer was originated from rhodamine B (555 nm excitation) covalently attached to the heads of phospholipids, whereas green image of inner portions of the lipobeads was originated from fluorescein-o-acrylate (488 nm excitation) covalently attached to the polymeric network. Two mechanisms of controlled load release are discussed for temperature sensitive hydrogel cores with lower and upper volume transition temperatures, respectively. Possible procedures for loading of curcumin or doxorubicin are proposed to develop versatile delivery systems of these curing agents.

Keywords: Characterization, Drugs, Membrane, Polymers & Plastics
Application Code: Biomedical
Methodology Code: Microscopy
Understanding the interfacial phenomenon, especially molecular transport, e.g., adsorption/desorption, diffusion and migration near a solid surface, is important since it is involved in both fundamental science and advanced applications. In chromatography, a plenty of surface modifications have been applied to change the interactions between samples and stationary phase to optimize the retention time to achieve the best separation. As a high temporal resolution technique, Fluorescence Correlation Microscopy (FCS) has provided an approach to study the particles/molecules dynamics in solution and near surface. However, due to the spatial resolution limit, it is challenging to differentiate diffusion within a small area from adsorption. Thus, a super-resolution technique, Stimulated Emission Depletion Microscopy (STED), has been used in combination with epi- and confocal fluorescence microscopy to study how particles/molecules retained in a C18 modified porous structure. In experiments, we found that the retention time of the particles in the pores does not correlate with the adsorption time of the particle on C18 surface. To fully understanding this phenomenon, different fractions of C18 and modifications have been studies. This study provides a new view to understand how particle-surface interaction affects molecular retention in porous media.

Keywords: Adsorption, Membrane, Microscopy, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Microscopy
The extracellular matrix is composed of a complex mesh of matrix protein and glycosaminoglycans heterogeneously decorated with sequestered biomolecular cues, which together with soluble cues, control cell fate and function. It has long been appreciated that traditional 2D cell culture plates poorly model native cell environments, which in turn can lead to unnatural cellular behavior. We have developed a suite of techniques to modify collagen-based 3D cell culture scaffolds to more accurately mimic the native mechanical and biomolecular complexity of the ECM. Using benzophenone (BP) mediated photoimmobilization we demonstrated functional patterning of vascular endothelial growth factor (VEGF), which induced spatial induction of endothelial cells towards a vascular fate and an overall increase in penetration into the scaffold. This photochemical approach to factor immobilization is limited in the depth of patterning by the opacity of collagen scaffolds. To alleviate this limitation, we have implemented a layering-based approach to create scaffolds that have biomolecular cue variation in all three spatial dimensions. We have utilized these layered scaffolds to controllably differentiate mesenchymal stem cells towards both a tendon and bone phenotype with an interfacial region in the middle mimicking the native tendon-bone interface—a challenging and important target for regenerative medicine. Overall, we have developed a generalizable toolbox to rationally modulate both the mechanical and biomolecular presentation of cues that more accurately recapitulate native environments for a range of advanced cell culture and tissue engineering applications.
Total holographic characterization uses holographic video microscopy to measure the size, refractive index and three-dimensional position of individual colloidal particles moving freely through their fluid medium. The detailed information derived from these measurements have applications in product development, quality assurance and manufacturing process control in industries as diverse as consumer products, food, cosmetics, medical diagnostics, pharmaceutical manufacturing, petrochemicals, among others. Based on the Lorenz-Mie theory of light scattering, holographic characterization yields a particle’s size to within a nanometer and its refractive index to within a part per thousand. These data are available for each particle in the microscope’s field of view and for each snapshot in the holographic video stream. No other particle characterization technique provides such a wealth of information. The analysis is fast enough that population statistics on tens of thousands of particles can be amassed in minutes. Holographic characterization therefore yields insights into the composition of inhomogeneous and multicomponent colloidal dispersions that cannot be obtained in any other way. Holographic tracking data, obtained at the same time, yields insights into individual colloidal particles’ responses to external forces, their interactions with bounding surfaces and their interactions with each other. In combination with optical micromanipulation, these measurements provide an unprecedented view into the stability and dynamics of colloidal dispersions.
Microscopy Techniques for Nanotechnology and Biomedical Applications

Super Resolution Microscopy to Characterize Surface Functional Group Heterogeneity of Activated Polymer Surfaces for Application in Nanoscale Electrophoresis

Currently, many lab-on-a-chip devices are fabricated in fused silica, which has a homogenous distribution of surface functional groups; however, fabrication of glass-based devices is costly and time-consuming. Whereas thermoplastics offer low cost, simple and scalable fabrication, and ease of functionalization via surface oxidation (UV/O\textsubscript{3} and O\textsubscript{2} plasma). Activation and characterization of thermoplastic surfaces is essential for many lab-on-a-chip applications, especially for electrophoretic separations in nanometer scale columns. We have previously characterized surface-confined carboxylic acids (COOHs) present on UV/O\textsubscript{3} modified cyclic olefin co-polymer (COC) and poly(methylmethacrylate) (PMMA). However, due to analytical limits, the density and spatial distribution of COOHs could not be determined, which is essential for evaluating non-plug like flow that may be generated with high surface charge heterogeneity. We have developed a new approach to characterize activated surfaces utilizing super resolution microscopy, specifically Stochastic Optical Reconstruction Microscopy (STORM). Surface COOH groups were generated via UV/O\textsubscript{3} or O\textsubscript{2} plasma treatment with the COOHs aminated and covalently labelled with a fluorescent marker and imaged by STORM. Both COC and PMMA were analyzed via STORM as a function of activation dosage. Results indicated a higher COOH density on COC substrates compared to PMMA with an optimal activation dosage. Also, high surface heterogeneity was noted. Furthermore, using COMSOL, the STORM images were mapped onto surfaces in three dimensions across nanochannels to simulate electroosmotic flow (EOF) for different functional group densities and uniformities. The results indicated that low density surface charges and non-uniform distributions introduced a non-plug like EOF. The effects of this non-plug like EOF profile will be discussed in the context of nanoscale electrophoresis.
One of the most common traditional methods for moisture specific analysis is Karl Fischer (KF) titration, in which iodine is reacted with water molecules to create an electrical current that can be measured. This has shown to be a reliable method of moisture-specific analysis, but it requires and consumes hazardous wet chemical reagents and delicate glassware and so is not a practical choice for many applications, industries and environments.

The Computrac® Vapor Pro® moisture specific Relative Humidity (RH) analyzer is an ideal replacement for a KF titrator. This technology thermally vaporizes water from a sample and sweeps it over a dry RH sensor using a dry carrier gas. Although other volatiles may also be present in the gas, the RH sensor is specific to only water molecules and will accurately and reliably detect moisture in virtually any type of product. No wet chemical reagents are required for this RH sensor method, making it safer, less prone to interferences, and cost-effective. In this paper, we demonstrate equivalency between RH and KF methods in plastics, food and pharmaceutical products while weighing the positive and negative aspects of each method.

Keywords: Analysis, Instrumentation, Polymers & Plastics, Thermal Analysis
Application Code: Polymers and Plastics
Methodology Code: Thermal Analysis
The integration of optics and fluidics at the microscale is a powerful tool and can be used for many biological applications. Amongst the ones being studied at present are single or multiple cell sensing [1,2], sorting [3], study of intra- and extracellular forces [4, 5] and spectroscopy (SERS) [6]. Although some of these techniques are quite mature, a major bottleneck is the complexity of system fabrication and assembly. Optofluidics is at present performed in large part with samples that are cumbersome to fabricate, expensive and require complex alignment procedures [7]. We here describe the fabrication procedure of an injection molded TOPAS polymer chip for optofluidic applications. Such chip allows simple assembly with commercially available waveguides, precise alignment and is suitable for fabrication in large batches [8]. Further advantages lie in the chemical and thermal resistance as well as optical transparency of TOPAS that allow on-chip processing and optical detection. Fabrication details as well as first optofluidic tests of both single and multiple cell manipulation will be shown.

### Abstract Text

Polyolefins are an important class of polymer that are used in common plastic materials. The most common polyolefins, polyethylene (PE) and polypropylene (PP) are often characterized from their pyrolysis products by Py-MS [1]. Nowadays the development of direct probe atmospheric pressure sources allow the direct analysis of these polymers [2]. The polymer deposited on a glass tip is desorbed with a hot nitrogen flux and ionized by nitrogen or helium plasma. These sources operate at atmospheric pressure, which imply a limited control of the ionization conditions. It was shown that side reactions could occur with species present in air such as O\(_2\) that may lead to the formation of oxidized compounds [3].

In this work ion mobility - mass spectrometry (IM-MS) and Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR) was used for the exhaustive characterization of the PP and PE pyrolysis ions produced using plasma-based atmospheric pressure ion sources. Both PP and PE yielded distribution of pyrolysis products presenting different number of unsaturation as shown by Lattimer but also different number of oxygen atoms. PP yielded mostly species presenting one and two oxygen atoms while PE yielded species presenting 0 to 7 oxygen atoms (Figure 1). From the FTMS data is was possible to obtain elemental composition of each ion allowing to examine extensively the different ion distributions whereas IM-MS yielded information on collision cross section allowing the differentiation of isomeric ions (Figure 2). In particular the ions produced from PP presented a lower CCS than those produced from PE.


### Keywords:
- Ion Cyclotron Resonance
- Mass Spectrometry
- Polymers & Plastics
- Pyrolysis
The Laser Flash or Light Flash Technique (LFA) is a well-known method for the determination of thermophysical properties of, for example, carbon materials and metals from below room temperature up to high-temperatures (e.g. -125[degree]C to 2800[degree]C). In this technique, the front surface of a plane-parallel, disk-shaped sample is heated by a short, high-energy pulse. The resulting temperature rise on the rear surface is measured versus time using an IR sensor. The advantages of the LFA technique are its short measuring times, easy sample preparation and high measuring accuracy. It is also a contactless and non-destructive measuring method. Simultaneous determination of the thermal diffusivity and the specific heat are possible. With knowledge of the material’s density, direct determination of the thermal conductivity is possible. Until recently, the LFA technique has mainly been used for well-conducting materials like carbon materials and metals. This poster will describe the newest developments and special sample holders which allow measurement of low-conducting materials such as polymers, composites, and liquids / powders. This poster will detail a new sample holder for low-viscosity liquids such as water, oils and resins (e.g. during curing). The newly designed sample holder consists of parts made of stainless steel combined with a PEEK ring which allows faster, less costly thermal conductivity measurements with high precision.

Keywords: Characterization, Materials Characterization, Materials Science, Polymers & Plastics
Application Code: Polymers and Plastics
Methodology Code: Other
Some lignin derivatives as methylated lignin, thiolignin and lignosulphonate were prepared and evaluated as reinforcing filler for rubber. They were incorporated with rubber and its ingredients through mixing process of rubber industry. The mixing was done on two roller with 460 mm in diameter, working distance 250 mm, speed of slow roll 16 rev./min. and gear ratio 2. The rheometric characteristics of the compounded rubber were measured using Monsanto oscillating Disc rheometer. The mechanical properties were tested according to the standard methods. The analytical data obtained from the tested properties were evaluated. It was found that, methylated lignin and thiolignin increase the optimum cure time and slightly decrease the rate of vulcanization. Lignosulphonate increases the rate of vulcanization as confirmed from the cure rate index. The physico mechanical properties illustrated that the presence of 10 phr from methylated lignin or thiolignin in the vulcanizates increases tensile strength and modulus at 200% of strain. The crosslinking densities were increased as indicated from the percentage of equilibrium swelling. The thermal stability of the investigated vulcanizates were determined by measuring the mechanical properties after thermal oxidative aging at 90 degree. The vulcanizates tolerated aging up to 7 days, also, resist the ultra violet light up to 10 days after subjecting to UV lamp of 200-300 nm.

Keywords: Characterization, Polymers & Plastics, Rheology, Rubber
Application Code: Polymers and Plastics
Methodology Code: Physical Measurements
Analyzing Chemical Composition of Rigid Polyurethane Foams Using FTIR Microscopy

The chemical composition of rigid polyurethane foams was studied using FTIR microscopy. Due to temperature gradients during the processing of rigid polyurethane (PU) foams, chemical gradients are observed across the radial axis creating a chemically heterogeneous composite. Furthermore, as you traverse across the cell micro-structure of these cellular materials, the chemical composition of the cell wall becomes distinct from the chemical composition of the gas filled cell nucleus. FTIR microscopy offers an ideal tool for studying variations in chemical composition at these microscopic levels. The chemical gradients in cross-link functional groups across the radial axis of a rigid PU foam in addition to residual starting materials such as isocyanate present in the foam is analyzed and presented.

Keywords: FTIR, Microscopy, Polymers & Plastics, Spectroscopy
Application Code: Polymers and Plastics
Methodology Code: Microscopy
Polymer materials and films impact our daily lives. Many characteristics of these materials have been enhanced by the addition of nanoparticles to provide unique solutions we see and touch every day. Weight reduction, safety, cost control and appearance in the automotive industry being only one application where polymers are an important materials. The determination of color and haze, (clarity) in automotive finishes and components such as windshields and other transparent or semi-transparent materials and polymer films impacts both appearance as well as safety and needs to be fully characterized. UV/Vis/NIR spectrophotometers equipped with a correctly designed 150 mm integrating sphere are a critical analytical platform necessary for the characterization of color and haze for both development as well as Quality Assurance testing needed for these materials as they become more complex.
Contaminants of are an important issue for plastic recycling activity. The control of incoming material and washing process is necessary to minimize this risk. Heracles system can be used to quantify very rapidly the residual solvents, such as benzene and toluene, and odorous compounds such as limonene in the incoming material and the finish product. The AroChemBase software can also be used to investigate the nature and odor threshold of unexpected contaminants. Plastic such as Polyethylene (PE), polypropylene (PP), High Density Polyethylene (HDPE) and polyethylene terephthalate (PET) are increasingly recycled. Recycled plastics face a higher risk of contamination and unwanted off-odors that could affect the organoleptic properties of the food they protect.

In this study, HERACLES fast gas chromatograph as used to detect the main contaminants of recycled plastics. This instrument features two metal columns of different polarities (non polar RXT-5 and slightly polar RXT-1701, length = 10m, diameter = 180µm, Restek) mounted in parallel and coupled to 2 Flame Ionization Detectors (FID). It allows headspace or liquid injection mode and also integrates a solid adsorbent trap thermo-regulated by Peltier cooler (0-260°C) for pre-concentration of light volatiles. Recycled HDPE resin was analyzed with or without standard additions. A standard mixture of n-alkanes (n-hexane to n-hexadecane) was analyzed to allow retention time conversion.

Calibrations were conducted using a mixture of chloroform, benzene, toluene, 4-vinyl-1-cyclohexene, trichloroethylene, limonene, methyl salicylate and benzophenone in methanol. The detection limits of the analytical method in plastic ranges from 0.5 µg/kg to 2 mg/kg depending on the compound and the potential contaminant molecules of plastic can be isolated in a chromatogram in less than 2 minutes.

**Keywords:** Contamination, Gas Chromatography, Polymers & Plastics

**Application Code:** Polymers and Plastics

**Methodology Code:** Gas Chromatography
Polymers and Plastics

Possible Mode of Action of Cyanuric-Phosphonate Derivatives as Flame Retardants on Cotton Fabric

Diethyl 4,6-dichloro-1,3,5-triazin-2-ylphosphonate and dimethyl (4,6-dichloro-1,3,5-triazin-2-yloxy)methylphosphonate are known to be very effective flame retardant (FR) additives for cotton fabrics. To understand their mechanism of action, an investigation of their thermal degradation on twill fabric was carried out in three steps: a) using the attenuated total reflection infrared (ATR-IR) spectroscopy to characterize the control and treated fabrics; b) employing the thermogravimetric analysis coupled with fourier transform infrared (TGA-FTIR) to study the evolved gases produced thermally; and c) applying the bond dissociation energy (BDE) to measure the strength of bonds in both FRs. Several novel features observed in the infrared spectra of materials include characteristic absorption peaks of phosphonate in the solid samples and of the methanol and ethanol in the released gases. Furthermore, the change in the enthalpy favors the cleavage of the phosphonate groups and chloride which lead to the possibility of the formation of cyanuric acid. These results reveal some distinctive details in the thermal degradation of the two compounds and they manifest their flame retardant nature on the substrate.

Keywords: Molecular Spectroscopy, Polymers & Plastics, Thermal Analysis, Volatile Organic Compounds

Application Code: Polymers and Plastics

Methodology Code: Molecular Spectroscopy
Microrheology Using DWS Spectroscopy for Gel Point Determination

Passive microrheology based on Diffusing Wave Spectroscopy is a new innovative technique for the study of the viscoelastic properties and the gel point transition of polymers and hydrogels. Passive microrheology consists of using micron sized particles to measure the local deformation of a sample resulting from the thermal energy, that is to say the Brownian motion.

DWS is an extension of the DLS technique to opaque media. The determination of the Mean Square Displacement (MSD) curve enables the complete characterization of the viscoelastic properties of a sample. The technique allows to measure particles displacement in a spatial range between 0.1 and 100 nm and a time scale between 10^-3 and 10^5 seconds.

Biopolymers have been introduced to a large scale of formulation not only in the food sector. Their unique properties in stabilization, structuration as well as texturation made them to extensively used components. Especially the gel formation capacity is of utmost interest.

This work presents a rescaling data processing known in rheology as time curing superposition [1-4], to determine precisely the gel point transition and gel strength parameters according to the Winter-Chabon-Criterion. Results will show the determination of the gel point versus time for carrageenan, gel point versus concentration for xanthan polymer, cross-linker concentration effect on the gel point.

Keywords: Instrumentation, Laser, Light Scattering, Polymers & Plastics
Application Code: Polymers and Plastics
Methodology Code: Other
The analysis of flavonoids in citrus species is often used for quality control and product authenticity because of their remarkable taste properties and variation in flavonoid profiles in different species and varieties of citrus (1,2). The most common technique is RPLC with UV/Vis detection or PDA detection. The challenge in this analysis is that flavonoids consist of dozens of structurally similar compounds, typically differing only in the degree of ring substitution, the type of substitution, and the type and degree of glycosylation. PDA detection lacks the selectivity in distinguishing the subtle structural difference in flavonoids. Mass spectrometry (MS) can provide better selectivity and better sensitivity for flavonoids analysis than the PDA does. However, the cost and the ease of use of MS instrument has always been a concern, especially for QC labs in manufacturing plants. The ACQUITY QDa Detector can provide mass spectral data with minimal tuning at affordable cost. It not only extends the sample detection to compounds with no UV or fluorescence chromophore but also improves the selectivity, which leads to a simpler and faster liquid chromatographic methods.

Here we report a fast and simple LC analysis of flavonoids (Naritutin, diosmin, hesperidin, didymin, sinensetin, nobiletin, tetramethoxyscutellarein, and tangeretin) with a Waters ACQUITY UPLC HSS T3 column (2.1x100 mm, 1.8 µm) using an ACQUITY UPLC H Class system and an ACQUITY PDA and an ACQUITY DQa detector. The chromatography run time, including the column equilibration, is 13 min. The analytical performance is comparable to the existing QC method.

Keywords: Analysis, Flavor/Essential Oil, HPLC Detection, Quality Control
Application Code: Quality/QA/QC
Methodology Code: Liquid Chromatography
Interactive Communication with Instrument to Realize Effective Analytical Workflow in the Laboratory

The laboratory in which instruments are installed and the office in which operators perform day-to-day operations are usually far apart from each other. Consequently, analysts spend a great deal of time moving between both locations.

Interactive communication with instrument improves productivity and promotes an advanced laboratory environment with operation capabilities from the built in touch panel and a smart device, thereby enabling a PC-free laboratory.

This feature enables operators to perform minimal operations to start an analysis via the instrument while the data acquisition is synchronized with Chromatography Data System. In addition, smart device such as a smartphone and a tablet PC spreads and is inflected as a simple multi-functional terminal. These information terminals create a new paradigm to start analysis and remotely monitoring system status and chromatograms without using any special software.

The interactive communication with instrument allows easy access to a system regardless of the operating environment, such as the operation of a system installed under a hood, in order to analyze highly active pharmaceutical ingredients.

The operations in the laboratory are minimized and performed free of errors. More efficient use of laboratory is realized.

In this study, the improvement of the usability and workflow to reduce the laboratory work and space by this new concept is demonstrated.

Keywords: Lab Management, Laboratory Automation, Laboratory Informatics, Software
Application Code: Laboratory Management
Methodology Code: Laboratory Informatics
## Abstract

Mechanical action micropipettes are ubiquitous in laboratories and are used for many routine tasks, including the quantitative measurement and dispensing of analytical samples and reagents. Concentrations of biological and chemical components in the prepared samples for assays and tests are volume-dependent and incorrectly performed pipetting steps will directly impact the transferred volumes, and hence, the test results. The design and construction of these pipettes render their performance susceptible to the technique and skills used by the operator of such devices.

This poster describes the basic principles related to an operator’s pipetting technique, and quantifies the errors induced by using improper techniques. For example, not pre-wetting the pipette tip may induce up to 1.5% error in the dispensed volume, which may be added to another 1.5% variation induced by the length of the pause before removing the pipette tip from the sample solution. Adding to the cumulative error in pipetting are inconsistencies in aspiration and dispense speeds and applied pressure on the plunger (up to 0.5%), heat transfer from the hands (up to 1.8%), improper immersion depth of the pipette tip into the sample (up to 1.3%), and the choice of the pipetting mode (up to 2.5%).

For obtaining reliable laboratory test results it is imperative that all pipette operators are consistently using the proper pipetting technique. Training pipette operators on using the correct pipetting technique ensures confidence in test results, and is equally critical in assay transfer situations or whenever results from different operators and laboratories need to be compared to each other.

### Keywords
- Instrumentation, Method Development, Quality Control, Sample Preparation

### Application Code
- General Interest

### Methodology Code
- Physical Measurements
Controlling Vapor Pressure the Key to Improved Loss-On-Drying Moisture Analysis

Moisture content provides valuable information about yield and quantity, making it an important measurement from a financial standpoint. The most common method for determining moisture content is loss-on-drying where a sample is weighed, dried, and weighed again to determine the water loss. Unfortunately, this methodology is plagued by multiple sources of variation such as: weighing errors, recording errors, lack of a dry reference, and error introduced by varying ambient conditions. Automation can eliminate many of these sources of variation, especially weighing and recording errors, while facilitating the simultaneous analysis of multiple samples. However, to address the issues of defining dryness and varying ambient conditions requires controlling the vapor pressure in the oven such that all samples are dried to a common endpoint, regardless of ambient conditions. Since evaporation ends when the vapor pressure of the sample is equal to the vapor pressure of the oven, it follows that the way to eliminate the variation that results from changing vapor pressure in the oven is to always control the vapor pressure to a pre-defined value. Then, dryness could be defined as the point when the sample equilibrates to the controlled vapor pressure in the oven, as indicated by constant weight. For this study, the moisture content of flour was determined under varying relative humidities (vapor pressures) with and without vapor pressure control to determine if controlling vapor pressure eliminated variation due to changing ambient humidities. It was determined that the samples analyzed while controlling vapor pressure did not significantly vary while those analyzed without vapor pressure control decreased as relative humidity increased. The results indicate that an automated loss-on-drying moisture content system that controls vapor pressure improves the reproducibility of moisture content analysis while providing a consistent “dry” reference.

**Keywords:** Food Science, Water

**Application Code:** Food Science

**Methodology Code:** Physical Measurements
Facilities often quantify noise and vibration during fit-out, comparing the levels with established criteria and making modifications to conform. This approach, while laudable, presumes that the noise and vibration remains constant over time, or that the processing equipment, itself, will not add noise or vibration.

In recent years monitoring systems for vibration and noise – portable packages incorporating accelerometers, microphones, analysis software and communications hardware -- have become increasingly available, providing real-time information that can be correlated with laboratory activities. For example, one manufacturer measured vibration signatures at several locations on his apparatus in an effort to explain an intermittent degradation in performance. At another facility a researcher found that acoustic noise, generated by his system, already well vibration isolated, generated small vibrations that compromised his results. With continuous, real time monitoring correlations can quickly be established.

Monitoring systems have become increasingly sophisticated, providing real-time information that can be posted to the internet and accessed from a variety of locations. Alarm levels can be implemented, both in terms of amplitude and spectral range. Warning lights, sirens, video monitoring, and email alerts can be incorporated to warn of exceedances, limit damage, and uncover faults.

Vibration and noise monitoring systems require knowledge of the appropriate sensors to employ, the problematic levels of vibration and noise, and the spectral range of importance to the specific process. Bringing this knowledge together with a flexible system architecture can, in a reasonably short time and at surprising low cost, provide quality control for environmental conditions often ignored.

Keywords: Data Analysis, Environmental Analysis, Laboratory, Quality Control
Application Code: Laboratory Management
Methodology Code: Physical Measurements
Odors are produced from a variety of natural and artificial sources. In this regard, odors can be considered characteristic of the emission source. Thus odors can be exploited as markers for identification. As such, odor recognition has become an attractive field, with numerous applications in environmental monitoring, biomedical diagnostics, quality control, and public safety. Incidentally, odors, which are constituted by complex mixtures of volatile organic compounds, can prove challenging targets for accurate identification using conventional techniques. In observance of this limitation, the development of novel gas sensing systems and materials, for identification of complex mixtures has garnered much interest in recent years. Herein we describe a simple method of odor recognition based on the use of QCM virtual sensor arrays. Each array consists of a single ionic liquid thin film immobilized onto the surface of the QCM-D transducer. Upon exposure to 10 different odors both closely related and distinct, measurements of frequency shift ($f$) were recorded at multiple harmonics to obtain odor specific sensing patterns. The multiple harmonic data were then evaluated using principal component analysis and discriminant analysis, which revealed that each of the virtual sensor arrays allowed odor recognition with extremely high accuracy.

This material is based upon work supported by the National Science Foundation Graduate Research Fellowship Program under grant number DGE-1247192; National Science Foundation under grant numbers, CHE-1243916 and CHE-1307611; and funds form the Phillip W. West Endowment to IMW.
**Session Title**: Quality/QA/QC and Laboratory Management  

**Abstract Title**: Determination of Flavonoids from Ginkgo Biloba Extract by Capillary Electrophoresis Mass Spectrometry Using a Sheath Liquid Interface  

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**Co-Author(s)**: Craig E. Lunte  

**Abstract Text**

High resolution and high specificity methods are desirable for quality control of herbal supplements and plant material in order to identify purity and origin. In this study, a laboratory-built sheath liquid capillary electrophoresis mass spectrometry (CE-MS) interface was fabricated using a small dimension CE capillary inserted through a mixing-tee to analyze 14 naturally occurring flavones, flavonols, flavonones, and their glycosides. Electrospray ionization was performed in negative ion mode and daughter ion scans were used to characterize the flavonoids of interest. The CE separation was carried out using a borate based buffer, which is commonly considered incompatible for CE-MS applications. The migration order was dependent on the extent of B-ring hydroxylation, conjugation of the aromatic rings, number of glycosidic functionalities, and ability of the flavonoid to form stable borate complexes. Parent ion scans yielded [M-H]-, except for cis-diol containing flavonoids, which were also detected as borate adducts. Daughter ion scans of these adducts and flavonoid glycosides typically produced deprotonated aglycone fragments, which are instrumental in flavonoid identification. This method was used to identify flavonoids from the ethanolic extracts of Ginkgo biloba herbal supplements.

**Keywords**: Capillary Electrophoresis, Electrospray, Mass Spectrometry, Method Development  
**Application Code**: Quality/QA/QC  
**Methodology Code**: Capillary Electrophoresis
All measurements are performed with a goal, ranging from the assessment of the compliance of a product with a specification to the characterization of a new material. The adequate fulfillment of this goal depends on the fitness of measurement uncertainty (MU) for its intended use. The assessment of the compliance of a gold alloy with a specification for gold content must be performed with a low uncertainty due to the price of this component. The characterization of a meteorite must be performed with an uncertainty small enough to distinguish their composition from other minerals. Therefore in setting the measurement requirements, in addition to specifying performance parameters such as recovery, repeatability and bias, it is also necessary to set a target value for the MU. The International Vocabulary of Metrology (http://www.bipm.org/vim), defines the “target uncertainty” as the “MU specified as an upper limit and decided on the basis of the intended use of measurement results”. Unfortunately only in a few cases does the specification and/or the legislation define the target measurement uncertainty required for compliance assessment. This communication will describe how to set the target MU to achieve the goals of the measurement.
Many pharmaceutical and biotech companies are moving towards an Electronic Laboratory Environment. These systems greatly improve the scientist's ability to locate specific data compared to traditional paper notebooks. The ability to quickly locate past experiments shortens turnaround time for addressing regulatory questions, provides evidence to protect intellectual property, and allows for improved compliance through software engineering. These systems can also allow users to access their experimental information remotely, and provides a framework for data sharing and collaboration across departments. Implementation of these systems is an interdisciplinary challenge requiring strong relationships between scientists, software engineers, and project management. Discussions must range from high-level system information to granular details of both science and code. In this presentation, a case study of a team’s planning and implementation of a flexible, sustainable, and efficient electronic laboratory environment for an Analytical Development department is described. In addition, some of the challenges faced and lessons learned will be presented.
Analysis of Pain Killers Dispensed in Kenya for Active Ingredients Using Gas Chromatography-Mass Spectrometry

In this study the quantity of Aspirin (acetylsalicylic acid), acetaminophen (4'-hydroxyacetanilide) and caffeine (3,7-dihydro-1,3,7-trimethyl-1 H-purine-2,6-dione) active ingredients in pain relieving drugs commonly dispensed in Kenya were determined using Gas-Chromatography-Mass spectrometry (GC-MS) coupled system. The results obtained in this work showed that acetaminophen in Panadol drug is comparable to the amount in Neladol and Elymol drugs (455-560 mg/tablet) while Hedex drug had the least value (24mg/tablet). The highest amount of Aspirin was found in Maramoja (937mg/tablet) followed by Hedex drug (790mg/tablet. The Aspirin content in Panadol, Elymol and Neladol drugs were below the limits of detection. Maramoja drug had the highest amount of caffeine (93mg/tablet) while Action drug had amount of caffeine of 80mg/tablet. The amount of caffeine in Panadol was 0.1 mg/tablet which was significantly different from those obtained for Maramoja and Action drugs. The difference between the experimental values obtained from this study and the amount indicated on the labels for each tablet by the manufacturers was highest for acetaminophen in P500(212mg/tablet) and lowest in Panadol (26mg/tablet).

Keywords: Gas Chromatography/Mass Spectrometry, GC-MS
Application Code: Quality/QA/QC
Methodology Code: Gas Chromatography/Mass Spectrometry
Simultaneous analysis of fat-soluble vitamins in foods is challenging due to their different properties and concentrations. A typical method involves extraction and saponification of fat, followed by high performance liquid chromatography (HPLC) with UV/Vis detection. After saponification the extracts can be analysed for vitamin A directly, but for vitamin D3, the extracts have to be cleaned on a semi-preparative chromatograph and concentrated. For this reason the vitamins A and D3 were analysed separately. The HPLC of these compounds suffers from a long runtime, slow equilibration and poor reproducibility.

As the extraction and saponification of separate vitamin A and D3 methods are identical, we investigated whether it is possible to apply Ultra-Performance Convergence Chromatography (UPC2) to analyse the extract for vitamin A and D3 in one chromatographic run.

UPC2 is a separation technique that uses supercritical carbon dioxide as the primary mobile phase. It takes advantage of sub-two micron particle chromatography columns, low viscosity of the CO2 and an advanced chromatography system. This offers an alternative chromatography method to traditional HPLC methods and improves the selectivity of this assay. It also generates much lower solvent wastes compared to conventional liquid chromatography. In this work, we report a method for analysis of vitamin A and D3 in vitamin premixes and concentrates in one analytical run without purification or dilution. The metrological properties of the UPC2 and advantages of the method compared to the HPLC are also discussed.

Keywords: Analysis, Method Development, Quality Control, Supercritical Fluid Chromatography
Application Code: Quality/QA/QC
Methodology Code: Liquid Chromatography
Quality/QA/QC and Laboratory Management

Abstract Title
Quality Comparisons of Prepared Formulations

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Abstract Text
Mobile phase, buffer and chemical solutions are prepared for analytical techniques such as HPLC, UV-VIS, LCMS, spectrophotometry and other analytical techniques. These formulations are derived from method development or various published methods. Most often they are made in-house by an instrument or analyst. Documentation points out the potential for errors with formulations made by these techniques. These formulations can result in poor quality, out of specification (OOS) results, and batch rejections. Root causes can be traced to lot to lot inconsistency, contamination and human error. A solution to many of these issues is to utilize commercially prepared formulations.

This poster will examine references to the problems of in-house blending and need for quality premixed formulations. Commercially prepared blends for use as mobile phases, buffers and other analytical work are a solution to these issues. Analytical results from various commercially prepared formulations will be presented as evidence to demonstrate quality, lot to lot consistency, and reliability over time. By utilizing prepared formulations a company can increase efficiencies and improve their analytical results. Honeywell LabReady™ Blends by Burdick & Jackson™ is an example of an available solution specifically suited to eliminate these problems and improve analysis quality.

Keywords: Chemical, HPLC, Laboratory, Sample Preparation
Application Code: Quality/QA/QC
Methodology Code: Sampling and Sample Preparation
A Fully Automated Universal Raman Scanner for Samples in Vial, SERS Plate, Tablet Form, Powder Form, 96 Microtiter Well Plate, or 384 Microtiter Well Plate

Conventional optical scanners are normally designed for dedicated or couple types of sample containers or sample holding tray. The application of those traditional optical scanners are operated without automated optical light focusing. This poster will introduce the design of an automated universal Raman scanner for 2ml and 5ml vials, drug tablets, solid powder, liquid, SERS plates, as well as 96 and 384 microtiter plates. The system features patented laser self-focusing technique for reproducibly obtaining the maximum Raman spectra intensity for the substance scanned. The fully automated design achieved <1% RSD for 100 times measurements of sample in solid form, powder form, gel form, liquid form etc. It is an ideal Raman scanner for fast substance molecular spectra identification and sample amount quantification. Examples for the QA of lipstick, drug, tea, counterfeits will be presented.

Keywords: High Throughput Chemical Analysis, Quality Control, Quantitative, Raman
Application Code: Quality/QA/QC
Methodology Code: Vibrational Spectroscopy
In x-ray fluorescence analysis (XRF), sample preparation is an important step in the analytical procedure. A borate fusion is frequently used for the determination of main components. At the present, there are several gas and electrical fusion systems on the market.

We have now developed a new automatic electrical furnace which overcomes the well-known problems and disadvantages of systems based on muffle furnaces.

The design consists of a lift bottom oven where the door is the bottom and it can be opened using a lift to load the sample. The crucible containing the sample and the mold are held by a rack, which is placed onto the lift by an autosampler. The homogenization of the sample inside the furnace is solved by external spinning at the bottom with a bidirectional motor.

The casting function is overtaken by the autosampler while the rack with the crucible is unloaded from the furnace.

The new system shows excellent precision and accuracy for all elements typically analyzed in oxidic materials like cements, gypsum, sands, iron ores, clays, geological samples, etc.

The closed design also helps to avoid the loss of volatile elements like sulfur, fluorine and chlorine.

Keywords: Sample Preparation, X-ray Fluorescence
Application Code: Quality/QA/QC
Methodology Code: X-ray Techniques
Quality/QA/QC and Laboratory Management

Determination of Brominated Vegetable Oil in Soft Drinks by UPC2-MS

Brominated vegetable oil (BVO) is often used as a weighting agent, or a solubility-transmitter for citrus oils and other lipophilic compounds in soft drinks and beverages. The US FDA has established a BVO limit at 15 ppm in finished beverages, while many countries in Europe, Asia, South America, and Australia, have banned its use in beverages. Analysis of BVO is rarely reported. Gas chromatography with mass spectrometry (GC-MS) has been proposed recently for the analysis of BVO in soft drinks and cocktail syrups. This GC-MS method requires tedious derivatization (or saponification) of BVO, and has a long run time (about 50 min).

UltraPerformance Convergence Chromatography™ (UPC2®) is a state-of-art supercritical fluid chromatography (SFC) that provides exceptional efficiency and speed of separation. It has been applied to a wide range of compounds, including VO, and has shown great benefits in selectivity, throughput, and ease-of-use. This work demonstrates a rapid and simple analysis of BVO in soft drinks and beverages using UPC2-MS. BVO was extracted and analyzed directly without any derivatization. The chromatography total run time was 9 min. The analytical method performance (limit of quantitation or LOQ, repeatability, linearity, and recovery) as well as the analysis of BVO in soft drinks and beverages are presented.

Keywords: Food Contaminants, Mass Spectrometry, Quality Control, Supercritical Fluid Chromatography

Application Code: Quality/QA/QC

Methodology Code: Other
The Mayo Clinic Metals Laboratory is a small, specialized lab that includes an ISO class 7 cleanroom for trace elemental analysis. The lab performs analyses for essential, heavy, and trace metals in biological samples, as well as kidney stone analysis. The Metals Laboratory currently has 9 ICP-MS, 2 ICP-MS with HPLC, 2 ICP-OES and 2 FT-IR instruments. The lab employs 26.3 FTE, and performs 49 different analyses on whole blood, serum, urine, plasma, tissues, and kidney stones. These analyses are comprised of 99 orderable tests, performed utilizing 24 different analytical SOPs. Staff utilization within the Metals Laboratory had not been previously mapped, therefore, scheduling and staffing was difficult and at times, suboptimal. A utilization model was created that allows the lab to determine current and future staffing needs based on test volumes, available FTE, days tests are performed, analytic time, and indirect time. The staff utilization model created allows the lab to balance the workload on a day to day and shift to shift basis.

Keywords: ICP-MS, Lab Management, Metals
Application Code: Laboratory Management
Methodology Code: Computers, Modeling and Simulation
Gamification is the use of game thinking and game mechanics in non-game contexts to engage users in solving problems. There are various forms of games that we all are familiar with from apps on our mobile devices to TV shows. The use of games and scenarios to engage learning, thinking and problem solving is not too distant from the skills sets, we as analytical chemists want from our students, to gain during their education. However game thinking or scenario based exercises are seldom used in the teaching of analytical practices and principles. I have utilized the principles of gaming as a means to provide real-life scenario based teaching to students but also to develop transferable skills and establish competitive spirit. All traits that are essential to enhance employability. We have utilized ‘The apprentice’ TV show as the model for our game and activity, where we got students to look into was the scientific evaluation and marketing of a new analytical HPLC column. Our show was fully supported by Phenomenex UK and students had to pitch their project to a panel of expects just like the actual show. Students learnt the importance of scientific communication and the need to learn about how to utilize their scientific knowledge in different environments like marketing and business. This exercise is now regarded by the students as the best highlight of their course and during my presentation I will give you an overview of the benefits and pitfall of gaming exercises in education and how the educational gaming we used improved student performance and engagement.

Keywords: Analysis, Chromatography, Data Analysis, Education
Application Code: General Interest
Methodology Code: Education/Teaching
The challenges in teaching undergraduate analytical chemistry are numerous and evolving. These challenges may relate to class size and time constraints, student engagement, and fostering higher order thinking skills, among many other aspects of teaching. In this presentation, I will describe how my colleagues and I are working to address some of these challenges in our courses through the use of guided-inquiry activities. For example, we have implemented a new hands-on laboratory activity where students build their own photometers from simple and economical components. The activity reinforces key concepts from lecture and makes those concepts tangible. It also challenges the “black box” mentality that can be a byproduct of sleekly designed and user-friendly instruments, provides an introduction to LabVIEW, and gives students a strong sense of satisfaction upon completion. In addition, we have recently developed an online applet that allows students to build virtual block diagrams of instruments by arranging and connecting images of components. The applet provides on-demand feedback to students so that they can tinker with instrument designs and sort out their own misconceptions about the form and function of those instruments. Another interactive applet for analytical method development is being designed, and is styled after the popular “Choose Your Own Adventure” series of books from the 1980s and 90s. Students are given a sample to analyze for a particular analyte and are guided through a series of decisions, with stepwise feedback, to arrive at one of several possible final analysis methods. Although we are in the early stages of implementation and assessment, we anticipate gains in the teaching-learning experience and improved critical thinking from students. Ultimately, we hope that these guided-inquiry strategies will be adopted by the analytical community at large.

Abstract Text

Keywords: Computers, Instrumentation, Teaching/Education, UV-VIS Absorbance/Luminescence
Application Code: General Interest
Methodology Code: Education/Teaching
Abstract Text

Analogical reasoning is ubiquitous to human cognition, and the use of analogies in teaching is a well-established pedagogical tool. We have developed an analogy to gas chromatography/mass spectrometry (GC/MS) analysis in order to explain the key concepts of the emerging technology of comprehensive two-dimensional gas chromatography/mass spectrometry (GCxGC/MS). A clear understanding of the principles of GCxGC/MS is needed by a growing number of people inside and outside of the separation science fields (such as mechanical engineers or computer scientists), and the use of this analogy in introductory workshops has been very useful in bridging the concepts in a domain that is more familiar to the student (source concepts) and the concepts in the unfamiliar domain (target concepts).

In this presentation we will explain the separation and identification technique of “namography/alphabetography”. Namography, like chromatography, is capable of separating people’s names by the length of letters in their name. Alphabetography, like mass spectrometry, is capable of identifying a person’s name by providing a histogram of the letters contained in the name. We will demonstrate the basic operations in GC/MS through this analogy using a sample of the names of the 44 US presidents, and will also discuss the need to use GCxGC/MS for complex samples. We will also present software development that was conducted in conjunction with the development of this example.

Keywords: Gas Chromatography/Mass Spectrometry, Teaching/Education

Application Code: General Interest

Methodology Code: Gas Chromatography/Mass Spectrometry
Our analytical chemistry laboratory is a research-based lab in which we monitor persistent organics, formaldehyde, chlorophyll, phosphate, metal ions, water hardness, chloride, and nitrate in a local wetland over the course of the term. Each method is introduced over a two-week period, with calibration in the first week and calibration and analysis in the second week. Data are compiled over the term and analyzed for trends as a culminating project of the laboratory. Our ecology laboratory simultaneously monitors ecological health of the wetland using surveys of plants, macroinvertebrates, and amphibians. Scientists in the two labs collaborate on data analysis and presentation. Undergraduate scientists are involved at every stage of the project, from sample collection through data presentation.

**Keywords:** Teaching/Education

**Application Code:** General Interest

**Methodology Code:** Education/Teaching
The inverted, or “flipped”, classroom model facilitates the implementation of active-learning in the teaching space. Realigning the traditional lecture so that it is introduced outside of the classroom environment, the newly acquired time in the classroom is filled with active-learning techniques. This hands-on approach to learning replaces the passive lecture-centered method with an active, personalized learning experience where students can direct the learning process based on their needs. With a strong emphasis on quantitative skills and reasoning, the Analytical Chemistry course is a natural fit for the inverted classroom model. While the essence of the inverted classroom is simply to deliver an active-learning experience while in the classroom, the Analytical Chemistry course at Elmira College relies heavily on various technological tools in order to enhance the learning experience for the student both in and outside the classroom. Video production software, quick response codes and interactive writing surfaces, among other technologies, will be discussed within the context of enhancing the analytical chemistry experience. Additionally, assessment in terms of both student perception and performance will also be discussed.

Keywords: Education, Teaching/Education
Application Code: Other
Methodology Code: Education/Teaching
Advanced Analytical Techniques for High Throughput Pharmaceutical Analysis

Development and Implementation of Innovative High-Throughput Screening and Analysis Solutions to Support Discovery and Development of Active Pharmaceutical Ingredients in the Pharmaceutical Industry

High-Throughput Automation, Analysis and Screening of complex mixtures are key capabilities offered by the Analytical Chemistry Speciality Team (ACST) to support drug discovery and development at Merck. ACST specializes in complex chemistry problem-solving through state-of-the-art innovative analytical chemistry solutions. Members of the team employ high-throughput assay development screening, definitive metabolite synthesis and elucidation, and stereochemical analysis; as well as identify process impurities via high-throughput tandem high-resolution mass spectrometry. Presentation will include discussion on novel advances in the area of high-throughput screening of 1536 nano-scale chemical reactions, development of an electrochemistry mass spectrometry technology for metabolite identification and synthesis, and macromolecule screening capability for analysis and purification. The development and implementation of these high-throughput analytical technologies has helped to ensure the highest quality of drug discovery and API development.

Keywords: Analysis, Automation, Bioanalytical, Characterization
Application Code: Process Analytical Chemistry
Methodology Code: Other
**Abstract Text**

Manipulating samples as droplets within microfluidic devices has emerged as an interesting approach for chemical analysis and screening. In segmented flow, one embodiment of this technology, nanoliter samples are manipulated in microfluidic channels as plugs separated by an immiscible fluid, such as air or fluorinated oil. These plugs serve as miniature test-tubes in which reactions can be performed at high throughput. Microfluidic tools have been developed to split, dilute, extract, and filter such plugs at rates up to 10 Hz. We have developed methods to analyze plug content by mass spectrometry (MS) and electrophoresis. Analysis rates of 5 samples/s by MS and 1 sample/s by microchip electrophoresis are possible. A natural application of this technology is high throughput screening for drug discovery. By coupling droplet manipulation with MS detection, it is possible to greatly reduce reagent consumption, to save on costs, and eliminate the need for fluorescent labels or coupled reactions, to prevent false readings. The technology and application to screens of deacetylase reactions and protein-protein interactions will be presented.

**Keywords:** Electrophoresis, Electrospray, High Throughput Chemical Analysis, Lab-on-a-Chip/Microfluidics

**Application Code:** High-Throughput Chemical Analysis

**Methodology Code:** Mass Spectrometry
Advanced Analytical Techniques for High Throughput Pharmaceutical Analysis

Addressing the Throughput Challenges of MS-Based Screening Using Various Front-End Automation Technologies

Assays that utilize MS for analysis offer direct, label-free assessment of relevant probe substrates; however, these methods have traditionally required liquid chromatography systems coupled with atmospheric pressure ionization mass spectrometry and have slow cycle times that are not amenable to high throughput screening in early drug discovery. Other available high-speed methodologies are often limited because of their use of labeled substrates, which are costly to synthesize and often yield different results than those obtained with physiologically relevant substrates downstream. In an effort to provide MS-based screening support with throughput speeds approaching those of plate-readers it is necessary to evaluate multiple platforms.

Recent advances in front-end technologies have resulted in significant improvement in the speed of MS-based analysis. First of all, multiplexing several fast-gradient HPLC systems and performing staggered injections onto the same MS has reduced the per-well cycle time to as low as 15-20 seconds. Another approach completely bypasses HPLC separation and instead performs direct online SPE with very fast robotics. With these systems, it is possible to achieve cycle times of 5 seconds per well. A 3rd approach attempts to utilize the very high speed MS analysis offered by matrix-free laser desorption/ionization (LDI) techniques. However, liquid assay samples have to be deposited onto the carrier prior to MS analysis, potentially shifting the bottleneck to sample preparation. More recently, acoustic nano-dispensing technology has been leveraged to “print” liquid samples directly onto the carrier prior to LDI analysis. The coupling of acoustic dispensing and LDI makes this technology amenable to post-reaction sample cassette analysis; allowing multiple reaction samples to be transferred into the same well, resulting in even faster throughput.

Applications of these platforms will be discussed in detail during this presentation.

Keywords: Bioanalytical, Laser Desorption, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry
Application Code: Drug Discovery
Methodology Code: Mass Spectrometry
This presentation will provide a vision of future automated testing procedures of solid oral drug product, and then discuss how current automated spectroscopic tablet analyzers enable us partially integrate and streamline final product testing process with speed and accuracy. In this study, the optical performance of an integrated tablet tester, TANDEM III that can measure tablet thickness, weight and near infra-red spectra in a sequence was evaluated against standalone spectrometers. Then, the tablet tester was connected directly to a tablet press in a pilot plant, allowing for automatic, nondestructive and real-time content uniformity analysis. Meantime, the integrated tablet tester was also connected to a data warehousing package to allow synergistic integration with other real-time process data. Two categories of development efforts, including automated equipment method development and traditional spectroscopic method development, deem necessary to enable a robust testing process. A more in depth discussion will illustrate the benefits and concerns that come with implementing Tandem from laboratory to pilot plant environments regarding:

1. the product formulation impact to the automated equipment method development;
2. the pilot plant environment influence on equipment automation;
and 3. the utilization of 3-D printing technologies to advance spectroscopic method development.

The automatic tablet tester provides both physical and chemical characteristics of pharmaceutical tablets, allowing both timely collection of process data and control of tableting process. Its high throughput and easy integration with digital control systems have greatly avoided overloading traditional analytical laboratory and led to a paradigm shift in the capability and capacity arrangement.

**Keywords:** Automation, Drugs, Near Infrared, Pharmaceutical

**Application Code:** Pharmaceutical

**Methodology Code:** Near Infrared
Reducing the Burden of Analytical Data Decision Making in High Throughput Parallel Synthesis and Route Scouting

Is having to manually curate analytical results, make routine determinations and add comments really necessary? Are there not better things we can be doing with our time?

Parallel synthesis strategies have become a key part of our product scale-up strategies in recent years by optimizing cost of manufacture, time to decision of the best scale-up route, allowing for greener chemical synthesis routes, reducing downstream product cleanup strategies and providing new routes of product patent protection. Moving to a matrix based optimization process, by default creates many new process bottlenecks, one of which is the interpretation of the analytical data. In visiting, talking and working with scientists from different laboratories around the globe, we remain surprised that the analytical results interpretation process is often performed sequentially with manual review of each analytical result and marked by hand to produce the final summary of results into the project teams. Is this really necessary?

Our presentation focuses on recent developments in software automation that allow for data from individual or multiple instruments to be automatically processed, standard process chemistry calculations to be made, and an automated evaluation of the analytical data for common problems that are present in chromatographic types of experiments using example case studies. By automating the majority of the analytical process has led to an overall time reduction of more than 50%.

Keywords: Liquid Chromatography/Mass Spectroscopy, Pharmaceutical, Process Analytical Chemistry
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
**Abstract Text**

Low Energy Ion Scattering (LEIS) [1] has been used for decades for the analysis of single crystals and "real world" surfaces. The latter application is, however, often hampered by the use of non-dedicated instruments which in many cases do not allow the utilization of the key feature LEIS to full extent: The quantitative elemental characterization of the outermost atomic layer.

Typically, the surface is bombarded with noble gas ions with an energy of 1 - 8 keV. By detecting the scattered ions under a well defined scattering angle and measuring their energy distribution, the composition of the surface can be derived from the peaks in the energy spectrum. The intensity is directly proportional to the surface coverage, while neutralization of the ions when penetrating the surface discriminates signal from deeper layers. This sub-surface signal can be evaluated separately to give insight into the composition a few nm below the surface.

Modern LEIS instrumentation is optimized to make these features available on many different kinds of samples, ranging from supported catalysts over self-assembled monolayers to thin films. Key for this is the dedicated energy analyzer, which is optimized for sensitivity to limit the required ion fluence and therefore avoid significant modification of the surface. It also allows choosing the optimum noble gas ion species to resolve the elements of interest. Furthermore the sample treatment after transport through air to remove atmospheric contamination has to be considered.

In this presentation, the fundamentals of the technique, the consequences for the instrumental design and the associated protocols for sample treatment will be covered. In the second part a number of applications will be shown, illustrating the importance of the outer surface of materials in many cases, as well as the benefit of the analysis using LEIS.

X-ray diffraction (XRD) is a powerful technique for characterizing crystalline materials. For polycrystalline materials such as powders, metal foils, sintered samples, finished parts, coatings and films, XRD can be used not only to quickly identify a material but also to quantify its phase composition, unit cell parameters, crystallite size, microstrain, and texture. Many of these properties can be studied in situ as a function of temperature. XRD can also be made surface sensitive using grazing incidence diffraction, which enables depth profiling of distances on the order of nanometers. Performing diffraction in the plane of the sample allows those crystal planes perpendicular to the surface to be characterized.

For films and materials that are not necessarily crystalline, X-ray scattering techniques can provide useful information. X-ray reflectivity (XRR) for example can be used to determine the thickness, density, and roughness of each layer present in any film. Small angle X-ray scattering (SAXS) can be used to characterize the average size and shape of nanoparticles or macromolecules in a matrix (liquid or solid) as well as the average pore size or other meso- or nano-characteristic distances present in partially ordered systems. After a brief overview of these techniques, a few specific examples will illustrate the wide range of materials for which X-ray diffraction and scattering can be useful.

Keywords: Materials Characterization, X-ray Diffraction
Application Code: Materials Science
Methodology Code: X-ray Techniques
Photoelectron spectroscopy (PES) has been one of the most utilized techniques for understanding of materials physics and chemistry since its development by Kai Siegbahn for which he received the 1981 Nobel Prize in Physics. In a photoelectron spectroscopy measurement, the kinetic energy of electrons emitted by the sample upon excitation by UV or X-ray photons is measured. This measured quantity is related to the binding energy of the electrons in the material and depends upon the electronic structure of the material. Using photoelectron spectroscopy, one can directly probe the valence electrons in the material, those with binding energy between approximately 0 and -25 eV. These are the electrons involved directly in chemical bonding and are the band states in solids. Measurements as a function of angle, Angle Resolved Photoelectron Spectroscopy (ARPES), allow experimenters to measure the band structure of crystalline solids for comparison with theoretical models. At higher binding energies, the core electrons are observed. These electrons are sensitive to the chemical state of the elements in the material. When the photopeaks are measured at high resolution, fitting of the spectral lines allows for the determination of the chemical bonding and oxidation state of the elements in a material. Using ARPES on the core level peaks, diffraction effects can be used to obtain the local atomic structure of elements in different chemical states. This talk will focus on using PES to elucidate problems of reaction chemistry with examples including chromatography, semiconductor devices, and the nuclear industry.

Keywords: Characterization, Electron Spectroscopy, Materials Science, Spectroscopy
Application Code: Materials Science
Methodology Code: X-ray Techniques
Laminated polymer structures are found in wide ranging applications from food packaging to a wide variety of industrial applications across many markets. The laminates present an analytical challenge due to their complex structure and the migration of species throughout the entire construction. Of particular importance is understanding the surface chemistry of the sealant film, which can significantly impact important properties like coefficient of friction, heat seal temperature and long term performance. Interlayer adhesion is often achieved through corona treatment of the bonding polyolefin surfaces. The stability of the surface chemistry after treatment can be modeled to predict long term performance. Here, an array of surface chemistry related analyses will be presented including SIMS, XPS and surface energy.

Abstract Text
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Keywords: Characterization, Consumer Products, Surface Analysis
Application Code: Polymers and Plastics
Methodology Code: Surface Analysis/Imaging
We have recently reported a series of new materials for liquid chromatography and for digital data storage. Our latest materials for liquid chromatography consist of carbon nanotube templated thin layer chromatography plates, and our new materials for digital data storage consist of nanofuses that can serve as memory elements. A variety of techniques have been applied to the characterization of these new materials. For example, X-ray photoelectron spectroscopy (XPS) has provided surface elemental analysis and oxidation state information about our new materials. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) has been used to complement the information obtained by XPS, providing additional chemical information about the surfaces of our materials. ToF-SIMS is a form of surface mass spectrometry. Spectroscopic ellipsometry (SE) has provided film thicknesses, optical constants, and roughnesses. Atomic force microscopy (AFM) has provided more direct measures of film roughnesses and thicknesses. Scanning electron microscopy (SEM), transmission electron microscopy (TEM), and helium ion microscopy (HIM) have been invaluable for surface imaging. Rutherford backscattering (RBS) has provided bulk (ca. 1 micron) views of our materials. X-ray diffraction has shown their crystal structures. The combination of the results from these techniques has allowed us to develop a reasonable understanding of our materials.

Keywords: Electron Spectroscopy, Materials Characterization, Surface Analysis, Thin Layer Chromatography
Application Code: Materials Science
Methodology Code: Surface Analysis/Imaging
The desire for decreasing detection limits and the comprehensive analysis of more complex samples (all possible constituents of a sample to be determined qualitatively and quantitatively) leads to increasingly sophisticated analysis platforms. Powerful analysis methods for such problems include the comprehensive two-dimensional gas and liquid chromatography (GC x GC and LCxLC), each coupled with advanced, high-resolution mass spectrometers. But even with the combination of these two high-performance separation methods, it is often not possible to analyze a complex sample in detail (food, tissue, plasma, plant, sewage sludge, etc.).

In 2000 EB Ledford wrote about the relatively new comprehensive two-dimensional gas chromatography "GCxGC has revealed that many samples are more complex than we had realized."

We propose that the introduction of another dimension of separation will reveal an even greater complexity of the samples. Through the use of LCxLC-IMS-qTOF-MS not only isobaric substances can be separated. Because of the higher peak capacity (LCxLC and IMS) the chemical noise is reduced and the limit of detection is much more better as without IMS.

Here we present the combination and potential of LCxLC and GCxGC with IMS-qTOF-MS.

Keywords: Gas Chromatography/Mass Spectrometry, Liquid Chromatography/Mass Spectroscopy
Application Code: General Interest
Methodology Code: Liquid Chromatography/Mass Spectrometry
Advances in Two-Dimensional Liquid Chromatography

Selecting a Suitable Column for the Second Dimension in Two-Dimensional Liquid Chromatography

Comprehensive on-line two-dimensional liquid chromatography (LCxLC) can produce considerably larger peak capacities compared to optimized one-dimensional separations in analysis times as short as 15 minutes. However, this result depends on using optimized second dimension cycle times ($t_c$) and achieving fractional coverages ($f_{cov}$) close to 1. Typically $t_c$ is at an optimum between 12 and 21 s. We have also shown that using carbon clad columns in the second dimension ($D$) produces $f_{cov}$ ranging from 0.58-0.63 relatively independent of the choice of the 1D bonded reversed phase column (e.g. C18, perfluorophenyl or polar embedded type phase). In this work, we compared the use of an C18 column to a carbon clad phase in the $D$. The sample and 1D columns were similar to those used in the prior study. The SB-C18 2D column produced $f_{cov}$ values ranging from 0.18-0.39. In contrast, the carbon clad 2D column produced $f_{cov}$ values ranging from 0.49-0.56. While $f_{cov}$ values using the carbon clad column in the $D$ are lower than previously observed, the carbon clad $D$ column increased the $f_{cov}$ values by a factor of 1.25 to 3 depending on the 1D column used.

Keywords: Liquid Chromatography, Liquid Chromatography/Mass Spectroscopy, Method Development, Pharma
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Polysorbate is commonly used in therapeutic monoclonal antibody (mAb) formulations to prevent protein aggregation and adsorption. Polysorbate may degrade through different pathways like hydrolysis and oxidation overtime and lose the surfactant function. The analysis of polysorbate in mAb formation is highly challenging, not only due to the heterogeneity of polysorbate component structures and no-UV chromophores, but also due to the significant interference from high concentration therapeutic proteins. To understand the stability of polysorbate and the degradation mechanism in mAb drug formulations, we used two dimensional UHPLC coupled with MS and charged aerosol detector (CAD) to analyze polysorbate 20 in different protein formulations. Orthogonal separation mechanisms such as mixed-mode, ion-exchange and reversed-phase chromatography were used in different dimensions to remove protein interference, as well as to separate the different components and degradation products of polysorbate 20. MS was used not only to identify the components, but also served as the third dimension to further separate polysorbate ester subspecies. We found some polysorbate 20 esters degrade faster than others, and the degradation rates are different with and without the presence of antibodies.
In this work we will discuss the results of recent simulations and experiments aimed at comparing one-dimensional (1D) and two-dimensional (2D) separations of moderately complex mixtures, such as those encountered in the analysis of an active pharmaceutical ingredient and its impurities. We are interested in the potential of 2D separations to simplify method development and reduce analysis time, when compared to 1D methods, because of the increased resolving power that can be realized from 2D separations under certain conditions. At this point in time users of 2D-LC are exploring a number of implementations of the technique, ranging from offline to online methods, and from single heartcut (LC-LC), multiple heartcut (mLC-LC), selective comprehensive (sLCxLC), and fully comprehensive (LCxLC). In the case of moderately complex mixtures (e.g., 10 to 50 components), it is not clear which of these 2D techniques will provide the best compromise of resolving power and ease of method development, implementation, and use. In previous work we simulated 1D, LCxLC, and the simplest possible implementation of sLCxLC separations and found that sLCxLC is predicted to provide analysis time savings of up to 70% compared to 1D and LCxLC for mixtures containing 20 compounds. In this presentation we will describe simulations and experiments involving more flexible implementations of mLC-LC and sLCxLC, and share our perspective for the potential of these techniques with respect to method development and ease of implementation and use.

Keywords: Bioanalytical, HPLC, Method Development, Pharmaceutical
Application Code: General Interest
Methodology Code: Liquid Chromatography
In the past decade, the advancement of two-dimensional (2D) chromatographic technologies has led to the development of instrumentations and methodologies for scientists to perform analysis and separation of complex materials. In general, 2D chromatographic separations offer higher resolving power than conventional one-dimensional (1D) chromatography. However, for preparative scale separations of 2D chromatography, the applications are limited due to three challenges: 1) the high flow rate of the chromatography in both first and second dimensions, 2) large volumes of solvent or large quantities of samples loading on to the columns, and 3) the compatibility of the large volume of solvent transferred from the first dimension to the second dimension. We have developed new methods and instrumentations for preparative 2D chromatography (2D LC-LC/MS and 2D SFC-SFC/MS) to improve the process of separation of complex pharmaceutical samples. Most recently, we have designed and developed the preparative 2D LC-SFC/MS system.

The presentation will discuss the instrumentation designs and applications of preparative 2D LC-LC/MS for the achiral-achiral purifications of complex samples and preparative 2D SFC-SFC/MS for achiral-chiral purification of enantiomers. The applications of case studies will demonstrate the utilities of 2D chromatography on a preparative scale to solve the separation challenges of complex pharmaceutical samples for drug discovery and development.

Keywords: Liquid Chromatography/Mass Spectroscopy, Pharmaceutical, Prep Chromatography, Supercritical Fluid
Application Code: Drug Discovery
Methodology Code: Liquid Chromatography/Mass Spectrometry
Analytical Methodologies to Detect Economic Adulteration and Monitor Food Safety and Quality

The detection and prevention of economically motivated adulteration (EMA) in foods is central to the U.S. Food and Drug Administration’s (FDA’s) mission to protect consumers. However, developing analytical protocols to combat EMA can be more challenging than producing methods to analyze trace contaminants. There can be multiple approaches used to adulterate a particular food commodity, and adulterants are often selected specifically to avoid detection. In addition, testing procedures must be able to provide sufficient evidence to distinguish between foods which have been economically adulterated, as opposed to results which are simply due to the natural variation in a given food commodity. This presentation will highlight the procedures that have recently been used by the FDA to detect EMA in a number of food commodities including pomegranate juice, honey, milk, palm oil, and lemon juice. The rationale in the selection of analytical protocols will be discussed, as well as some unique and unexpected challenges that resulted from testing these products.

Keywords: Beverage, Food Identification, HPLC, Liquid Chromatography/Mass Spectroscopy

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Date: Thursday, March 12, 2015 - Morning
Time: 08:35 AM
Room: 261
Economically Motivated Adulteration (EMA) is a criminal activity, a fraud economically motivated. The objective is essentially that the amount of adulterant added to food must bring some economic gain, as seen during the melamine scandal in infant formula that surfaced in China in 2008. From an industry perspective, it is pivotal to understand the potential vulnerabilities of the different raw materials to economic adulteration and to assess the business risk. Raw material vulnerabilities are dependent on several parameters such as the raw material composition, level of processing (e.g. thermal treatment) and the criteria used to set the price (payment criteria). Reviews of past cases of raw material adulteration definitely help to provide complementary information on the sensitivity of each raw material to economic adulteration. In the case of beef for example vulnerabilities may include species substitution or adulteration with non-authorized preservatives.

Ensuring authenticity of raw materials in an efficient and lean way can only be achieved by combining appropriate mitigation strategies. These can vary and include the implementation of multiple analytical approaches, raw material procurement traceability, supplier audits, specification management, etc. An approach to evaluate raw material risk of adulteration will be presented, that includes how to establish priorities and to mitigate risk. One of the major difficulties and challenges in addressing EMA is the availability of suitable analytical methods to determine food adulteration, keeping in mind that the adulterant is not always known upfront. Analytical techniques used must encompass both targeted and non-targeted methods, keeping pace with continuously evolving fraudulent practices. An overview of current methods implemented at both the raw material collection centres and factory gate (e.g. FT-IR) are presented as well as more sophisticated laboratory methods (e.g. LC-MS/MS, PCR, NMR).
Olive oil is one of the most adulterated food products of the world due to its relatively low production and higher prices as compared to vegetable and seed oils. The study from UC Davis Olive Center in 2010 and 2011 showed that more than 65% of extra virgin olive oil sold at the US supermarkets did not meet the USDA “extra virgin” grade standards. The findings from this study received more than one thousand media coverage worldwide, including the New York Times, CNN, The Dr. Oz Show and CBS News. While these reports have helped to raise awareness in consumers and food buyers, there are a lot of challenges ahead in developing more efficient analytical methods and establishing better standards. In this talk, we will discuss about these challenges and recent analytical advancements on the authentication and adulteration of olive oil.
Coconut water is one of the fastest growing categories of fruit juice beverage in the US market. Very little has been published as yet regarding the composition of coconut water, providing an opportunity for its economically motivated adulteration. The presentation will discuss work we have done on the composition of coconut water and on how that information can be used for assessing the purity and authenticity of coconut water products.
Economic adulteration of extra virgin olive oil (EVOO), the highest grade of olive oil, is a threat to its authenticity. Cases of fraud of EVOO have long been reported by the media and in the scientific literature. To meet the EVOO Standard, an olive oil must meet chemical and organoleptic parameters. While FDA has jurisdiction over deceptive label declarations often found with adulterated EVOO, the Agency is also mandated (according to FSMA, SEC. 106) with the protection of the US public against the intentional adulteration of foods. The most serious issue facing regulatory agencies has been the relative ease to adulterate EVOO with lower grade olive oils or seed or nut oils while meeting the physical and chemical property limits of various established standards. While the FDA does not have regulations for addressing authenticity, quality, and purity of olive oils, the USDA and various other standards specify quality and purity criteria that include organoleptic characteristics, and designate specific official methods of analysis to be used as reference methods in determining the grade, quality, and purity of olive oils. The USDA standards also state that alternative methods may be used, provided they give equivalent results. NIR spectroscopy (using benchtop spectrometers and/or portable analyzers) and univariate and/or multivariate statistical analyses were applied to the rapid screening of 93 commercial EVOO purchased locally. Other untargeted and targeted complementary analytical tools and/or discrimination algorithms were also used. Depending on analytical methodology used approximately 5-20% of the oils were flagged as outliers. Adulteration at 10% and 20% (w/w) of an authentic EVOO reference with 10 different foreign oils, including palm olein and hazelnut oil, was also detected.

**Keywords:** Chemometrics, Near Infrared

**Application Code:** Food Identification

**Methodology Code:** Vibrational Spectroscopy
The folding, structure, and function of membrane proteins are often influenced by their surrounding environment but lipid interactions are often difficult to characterize. As a step towards understanding these interactions, I will describe an approach that we have developed to measure resistance to unfolding in the gas phase to rank the effects of lipid binding to membrane protein complexes. First, we had to overcome the tendency for membrane proteins to unfold in the gas phase, due to the activation needed to emerge these complexes from detergent micelles. After establishing the conditions that preserve the folded state of membrane proteins, as assessed by ion mobility MS, we selected three membrane proteins anticipated to respond differently to the lipid environment: the mechanosensitive channel, aquaporin Z, and the ammonia channel. We then established conditions whereby we could resolve individual phospholipid-bound states of membrane proteins, in solutions containing vast excesses of detergent and lipids, and then ranked bound lipids based on their ability to prevent gas-phase unfolding. Combining our IM-MS data for the three protein complexes with molecular dynamic (MD) simulations we identified probable lipid binding sites. Phosphatidylglycerol, identified as significantly stabilizing the ammonium channel, prompted us to determine its crystal structure in this lipid environment. The 2.3Å resolution structure that we determined reveals bound lipids in one of the sites predicted using MD simulations filtered by IM-MS measurements and, by comparison with previous structures, distinct conformational changes induced by lipid binding that reposition residues to interact with the lipid bilayer. Overall our results allowed us to conclude that resistance to unfolding correlates directly with specific lipid-binding events that not only modulate membrane protein stability but may also induce conformational change.

Keywords: Bioanalytical, Lipids, Mass Spectrometry, X-ray Diffraction
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
It is known that elevated cholesterol promotes amyloidogenesis, but the mechanism has not previously been clear. In this study the structure of the transmembrane C-terminal domain of the amyloid precursor protein (C99) was determined using NMR spectroscopy and found to have several surprising features to provide insight into how this protein is proteolytically processed in the pathway to formation of amyloid-beta, which is closely associated with the etiology of Alzheimer’s disease. It was also discovered that C99 is a cholesterol binding protein. Based on this and additional data, we propose a unifying model for how cholesterol promotes the amyloidogenic pathway. We then tested the hypothesis that C99 has an intrinsic affinity for raft-like liquid ordered (Lo) domains and that its domain preference is regulated by cholesterol binding. Fluorescence microscopy shows that C99 undergoes efficient partitioning into Lo domains relative to the co-existing liquid disordered (Ld) phase in giant unilamellar vesicles, establishing C99 as the transmembrane protein with the highest affinity for Lo domains known to date. This is reversed upon mutating residues that are critical for cholesterol binding to C99, indicating that binding of this sterol is a critical determinant of the phase preference of the protein. Addition of a raft-breaking sterol that can compete with cholesterol for binding to C99 shifts partitioning away from the Lo phase. These findings identify cholesterol binding as a novel mechanism that controls the raft targeting of C99 and suggest that pharmacological inhibition of this process could potentially be exploited as a therapeutic approach to avoid or treat Alzheimer’s disease.

This work represents collaboration with the lab of Prof. Anne Kenworthy, also of Vanderbilt University. This work was supported by NIGMS and the Alzheimer’s Association.

**Keywords:** Lipids, Magnetic Resonance, Membrane, Protein

**Application Code:** Biomedical

**Methodology Code:** Magnetic Resonance
Peripherin and its homologue ROM1 are retina-specific members of the tetraspanin family of integral membrane proteins required for the morphogenesis and maintenance of photoreceptor outer segments, regions that collect light stimuli. Over 100 pathogenic mutations in peripherin cause inherited rod- and cone-related dystrophies in humans. Peripherin and ROM1 interact in vivo and are predicted to form a core heterotetrameric complex capable of creating higher order oligomers. However, structural analysis of tetraspanin proteins has been hampered by their resistance to crystallization. Here we present a simplified methodology for high-yield purification of peripherin/ROM1 from bovine retinas which permitted its biochemical and biophysical characterization. Using size exclusion chromatography and blue native gel electrophoresis, we confirmed that the core native peripherin/ROM1 complex exists as a tetramer. Peripherin, but not ROM1, is glycosylated and we examined its glycosylation site and glycan composition by LC MS/MS. A novel form of native mass spectrometry was used to analyze the complex in detergent micelles, demonstrating its tetrameric state. Our electron microscopy-generated structure solved to 18 Å displayed the tetramer as an elongated structure with an apparent 2-fold symmetry. Lastly, we demonstrate that peripherin/ROM1 tetramers induce membrane curvature when reconstituted in lipid vesicles. These results provide critical insights into this key retinal component with a poorly defined function.

Keywords: Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry, Proteomics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Mass Spectrometry
Analytical Technologies for the Structural Characterization of Integral Membrane Proteins

Methodologies and Analytical Approaches for the Crystallization and Structure Determination of G Protein-Coupled Receptors

Due to their strategic location at the cell surface, integral membrane proteins (IMPs) play a crucial role in the regulation of cellular processes making them important and attractive pharmaceutical drug targets. Lead optimization of drug compounds can be accelerated with the availability of IMP co-crystal structures, but this has traditionally been hampered due to the numerous challenges associated with their low heterologous expression and low intrinsic stability in detergent micelles. G protein-coupled receptors (GPCRs) present added challenges because they are conformational flexibility, making it difficult to crystallize these receptors in a single stable conformation. The recent advancements in methodologies and analytical approaches that have enabled a number of groups to circumvent the obstacles to GPCR crystallization and structure determination will be presented.

Supported by JCIMPT grant: P50 GM073197

Keywords: Biopharmaceutical, Characterization, Drug Discovery, X-ray Diffraction

Application Code: Drug Discovery

Methodology Code: X-ray Techniques
Membrane proteins currently represent approximately half of all potential therapeutic targets and are involved in many biological functions. Biophysical characterization approaches such as X-ray crystallography and NMR are challenging due to poor expression levels of the membrane protein and also protein solubility. Native membrane protein analysis by mass spectrometry (MS) is an attractive alternative, however, to date has remained very challenging. A nanodisc, as the name implies are discoidal structures comprising of two membrane scaffold proteins acting as a belt, holding together a lipid bilayer, made up of around 160 phospholipids depending on MS-activating voltage used. As we demonstrate herein, the nanodisc is amenable to native MS and ion mobility analysis.

Upon mild ion activation in the atmosphere/vacuum interface region, a broad heterogeneous charge state distribution can be observed on QTOF, OrbiTrap and FT-ICR MS systems. The heterogeneity is attributed to varying levels of phospholipid incorporation into the nanodisc. However, significantly lower source activation conditions are required on the FT-ICR MS instrument to obtain well resolved spectrum in the high m/z region. This has interesting implications for native ion desolvation and will be discussed further in the context of instrument vacuum and ion residence times.

Quadrupole ion selection, followed by mild collisional activation conditions, were performed on both instruments, resulting in a much simplified empty nanodisc spectrum.

Atomic force microscopy images show individual nanodisks absorbed on the surface, multiple overlapping and multiple stacked (double) discs. The dimensions of the disc are approximately 20 nm in diameter and thickness approx. 1.5nm. The travelling wave and RF-confining drift-tube dimension measurements are consistent with the AFM measured dimensions, suggesting that the fidelity of the gas-phase and solution phase structures have been maintained.

Keywords: Biopharmaceutical, Drug Discovery, Mass Spectrometry, Time of Flight MS
Application Code: Bioanalytical
Methodology Code: Physical Measurements
Wearable sensors have garnered considerable recent interest owing to their tremendous promise for a wide range of healthcare, military, sport and wellness applications [1]. Such sensing platforms provide new avenues to continuously monitor individuals and can thus tender crucial information regarding a wearer’s health and performance in real time. This presentation will discuss recent developments in the field of wearable sensors ad biofuel cells (BFC), integrated directly onto both textile materials and on the epidermis for various non-invasive monitoring and energy-harvesting applications. The preparation and characterization of such textile-based and skin-worn electrochemical sensors and BFC will be described, along with their performance and the influence of the stretching and bending. Such mechanical stress studies indicate that tattoo and textile-based printed electrochemical sensors survive large deformations. Technical challenges and prospects for using textile- and tattoo-based electrochemical sensors for monitoring the wearer’s health, fitness, or surrounding environment will be discussed, along with several demonstrations and prospects for future healthcare and sport applications.

REFERENCES

Keywords: Biosensors, Electrodes
Application Code: Bioanalytical
Methodology Code: Electrochemistry
A need exists to develop simple, low-cost methods for pollutant sampling and quantification to support both health-based research (i.e., epidemiology) and citizen-based science. Our group has developed alternative approaches for metals speciation using paper-based microfluidic devices. Traditional and paper-based microfluidics are poised to overcome many issues associated with chemical analysis of metals in air, water, and soil, namely: cost, ease-of-use, and sensitivity. Results can be read using either the naked eye or simple, low-cost instrumentation (i.e., a handheld or mobile device). Such assays cost only a few dollars and can be conducted by virtually anyone after simple training. We have developed microfluidic assays on paper to measure reactive oxygen species and metals (Fe, Ni, Cu, Cr, Pb, Cd) in air and water using a combination of colorimetric and electrochemical detection motifs. Detection limits of 0.1 [micro]g (1-5 ppm solution levels) were achieved for colorimetric assays (appropriate for occupational exposures) while electrochemical assays provided detection limits of 0.1 ng (1 ppb solution levels; appropriate for environmental exposures). We have also validated these techniques against standard reference methods with relatively good agreement. To complement these analytic techniques, our group has also developed a series of low-weight, low-burden sampling devices. These devices feature micropumps that provide silent operation and a leap in energy efficiency, allowing significant reductions to size, weight, and cost. Initial prototypes are hand-held (or wearable, in the case of air monitoring), weigh less than 150g, and cost about ten times less than the current state-of-the-art samplers.

Keywords: Air, Instrumentation, Monitoring, Water
Application Code: Environmental
Methodology Code: Microfluidics/Lab-on-a-Chip
Emerging Technologies for Point-of-Care Biomonitoring

Point-of-Care Metal Exposure Assessment Using Electrochemical Microsensors

Metals are ubiquitous in the environment and have long been recognized to pose significant threats to human health. Blood lead (Pb) has been consistently associated with deficits in IQ and academic achievement in numerous controlled studies. Manganese (Mn) is an essential element, yet neurotoxic in excess, capable of crossing the blood-brain barrier and accumulating in the brain. Current approaches for measuring such exposures suffer from high costs including extensive labor, equipment and time-consuming laboratory procedures, and often, demonstrate long turnaround times. Our group is addressing this need by developing point-of-care sensors for assessment of metal exposure by integrating the electroanalytical techniques of stripping voltammetry with microfluidics. While anodic stripping of Pb has been well-reported in literature, stripping analysis of Mn on microscale remains a critical challenge due to the strong negative potential of its stripping peak. Necessity for low limits of detection, high reproducibility, and low (disposable) sensor costs present additional challenges. This talk will discuss our recent results that show feasibility of measuring Mn and Pb metals with point-of-care sensors. Our goal is to demonstrate rapid, point-of-care, multi-analyte assessment of Mn and Pb metals in a finger prick of blood.

Keywords: Electrochemistry, Electrodes, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Electrochemistry
We are developing screening methods that use microchip electrophoresis to determine quantitative differences in the serum N-glycan profiles of disease-free individuals and patients with cancer. Electrophoretic analysis easily resolves glycan structures and their isomers and provides complementary information to mass spectrometric (MS) methods. N-glycans are enzymatically cleaved from glycoproteins in serum and derivatized with 8-aminopyrene-1,3,6-trisulfonic acid to impart charge needed for electrophoresis and a fluorescent label for detection. For each sample, an N-glycan profile is generated from the electrophoretic analysis, and statistical analyses reveal differences among the profiles of the various sample groups. We have evaluated samples from esophageal adenocarcinoma, ovarian cancer, and colorectal cancer, and the serum samples from each type of cancer are readily distinguished from the samples of disease-free individuals. Through direct comparison of electrophoretic mobilities with MALDI-MS data and standard addition with N-glycans from common glycoproteins, we have identified a number of the N-glycans that contribute to the differences in disease states.

**Keywords:** Bioanalytical, Biological Samples, Lab-on-a-Chip/Microfluidics

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Magnesium and its alloys exhibit properties such as high strength, light weight, and slow corrosion in aqueous environment that make them promising candidates for the development of biodegradable metallic implant materials for bone repair, stents and other medical applications. Electrochemical sensors can be used to monitor the corrosion process of magnesium and its alloys by measuring the concentration of the reaction products magnesium ion, hydroxyl ion and hydrogen gas. The permeability of hydrogen gas through skin allows direct monitoring of corrosion of an alloy implanted beneath the skin by detecting hydrogen gas at the skin surface. An amperometric hydrogen gas sensor was used to map hydrogen concentration in the vicinity of an implanted magnesium alloy by placing the sensor tip adjacent to the skin to measure the hydrogen passing through the skin. The level of hydrogen detected is a measure of the corrosion rate of the implanted sample. Biomedical applications for this novel use of hydrogen sensing will be discussed.

Keywords: Bioanalytical, Electrochemistry, Sensors
Application Code: Biomedical
Methodology Code: Sensors
The recent revolution in nucleic acid sequencing and other methodologies permitting comprehensive, molecular-level characterization of biological samples is on the verge of transforming patient care to an extent not seen since the late 19th century. The initial assembly and continuous refinement of the reference human genome, together with a dramatic drop in the cost of sequencing, have enabled clinical applications that include not only the rapid identification of germline mutations in rare Mendelian diseases but also the detection of tumor- and disease stage-specific mutations in cancer and the discovery of genomic variants underlying common diseases with complex patterns of inheritance. The promise of this transformation is that it will lead to better understanding of disease pathogenesis and risks, more accurate prognostication and assessment of response to therapy and, most importantly, to a new standard in medicine wherein individual patients, rather than disease entities diagnosed by conventional approaches, are matched with specific therapeutic options. However, the genomic revolution also poses several new challenges including conceptual, technological and ethical hurdles. Arguably one of the greatest challenge is represented by the biological problem of how the information ‘hard-wired’ in the genomic sequence limits and regulates its own expression under the influence of signals from the environment in normal and diseased cells and the related technological problem of how this complex control system—termed ‘epigenetic’ for its role in regulating gene expression ‘above and beyond’ changes in DNA sequence—can be interrogated and modulated for the purpose of clinical diagnosis and therapy. This talk will review key elements of epigenetic control, currently available epigenetic methodology and highlight critical barriers to applying these concepts and techniques to individualized medicine. Supported by the Mayo Clinic Center for Individualized Medicine.
This presentation will describe the fabrication and use of cross-sectional size-adjustable elastomeric channels to linearize and image single chromatin fibers. These normally-closed channels are fabricated by controlled fracture of brittle films sandwiched between poly(dimethylsiloxane) (PDMS). Arrays of these crack-based conduits with defined spacing between channels are created by introducing flaw-shielding structures and applying a tensile strain that matches the channel spacing desired. Crack depth is controlled by using brittle films with relatively small mechanical property mismatch with the bulk substrate. By controlling thickness of the brittle film, channels can be created with widths and depths of tens of micrometers down to nanometers. The nanometer sized channels are useful for linearization of single chromatin fibers. These channels are loaded with chromatin by simply stretching open the normally-closed channels. The wider channel openings, coupled with the associated inflow of fluids enable easy biopolymer loading without use of any other fluidic pumping or electrophoresis. Once inside the channel, the biopolymers can be linearized by releasing the tensile strain to narrow the channels. The combined hydrodynamic fluid flow together with nanoconfinement aids in linearizing, then trapping the linearized biopolymers. This method is gentle enough to distinguish differences between chromatin reconstituted with and without histone H1 and is also sufficiently forceful to enable linearization of lambda DNA up to ~97% of its contour length. This presentation will focus on application to multi-color analysis of histone modifications.
This talk describes a streamlined DNA methylation detection platform that utilizes silica superparamagnetic particles to improve the processing of circulating DNA in serum/plasma and quantum dots to enhance methylation detection. In addition, a magnetic droplet platform of DNA sample preparation to further improve the sensitivity of methylation detection is also discussed.

**Keywords:** Bioanalytical, Biosensors, Lab-on-a-Chip/Microfluidics

**Application Code:** Nanotechnology

**Methodology Code:** Sampling and Sample Preparation
Epigenetic mechanisms such as histone post-translational modifications (PTMs), small non-coding RNAs and DNA methylation play crucial roles in the establishment and induction of gene expression patterns that regulate several aspects of cellular biology. While it is appreciated that activation of cells and thus signaling pathways affect gene expression, exactly how this is accomplished is not well understood. We will present data on new approaches to determine how signal transduction pathways make their way into the nucleus to affect histone modifications and gene expression patterns using chemical biology and quantitative proteomics. These approaches involve new isotopic labeling methods that specifically labels phosphorylated proteins, as well as a covalent labeling method to affinity purify out phosphorylated proteins on chromatin associated nucleosomes, with an emphasis on the EGF signaling pathway.

Keywords: Genomics, Mass Spectrometry, Proteomics
Application Code: Genomics, Proteomics and Other 'Oomics
Methodology Code: Mass Spectrometry
Epigenomic tests, and in particular assays that probe protein-DNA interactions, are an emerging tool in the development of personalized cancer treatment strategies. Chromatin immunoprecipitation (ChIP) is the gold standard for analyzing protein-DNA interactions; however, conventional ChIP is limited in its clinical utility as it is extremely laborious and requires large cellular input (1-10 million cells). These requirements significantly reduce the applicability of ChIP in a clinical setting—particularly when minimal sample is available, such as in the analysis of tumor biopsies, stem cells, or circulating tumor cells.

Microfluidic devices offer many attractive benefits over traditional macro-scale methods including reduced volume requirements, parallelization capability, and automated operation, which make them particularly well-suited to sample-constrained epigenomic analyses. We are developing a powerful and versatile, droplet microfluidics-based platform that will allow for automated ChIP analyses. Our platform incorporates every major step in the ChIP workflow into an automated device, including cell lysis, chromatin digestion, immunocapture, and DNA purification. Importantly, these processes will be carried out at the single cell level, which promises to provide unique insights into epigenomic tumor heterogeneity. Beyond single cells, the unprecedented ability to handle samples of variable input will also facilitate robust validation against traditional ChIP assays to demonstrate broad genomic coverage. Taken together, we feel that the resulting platform will be a powerful new tool that helps enable the translation of epigenomic insight into individualized cancer treatment at the point of care.
Forensic Analysis in the Lab and Crime Scene

Vibrational Spectroscopy for Gunshot Residue Analysis

Vibrational spectroscopy, attenuated total reflection (ATR) FTIR and Raman spectroscopy, has numerous applications in forensic chemistry providing confirmatory identification of analytes. The technique is non-destructive, rapid and requires little or no sample preparation. Furthermore, portable ATR FTIR and Raman spectrometers are readily available allowing for crime scene accessibility. Vibrational spectroscopy offers several advantages over the current methodology for gunshot residue (GSR) analysis. The technique was shown to detect components from both the organic and inorganic constituents of GSR. This is contrary to current GSR elemental analysis methods which rely solely on the detection of the heavy metals (lead, barium and antimony). This is problematic since environmental concerns have led to the increased popularity in heavy metal free or “green” ammunition that severely hindered the identification of GSR by the current elemental analysis techniques. We will discuss a recent development in the application vibrational spectroscopy for detection and identification of GSR.

Keywords: Chemometrics, Forensics, Trace Analysis, Vibrational Spectroscopy
Recent applications of new and existing microextraction sampling methods developed for the classification of a wide variety of forensic specimens are being presented. Volatile organic compounds from individuals have been employed to differentiate persons. This type of evidence is also capable of providing information about an individual’s shared traits such as age and gender and may prove useful in instances when other trace evidence such as DNA is unavailable. The classification power of this technique will be presented from three different ethnic groups sampled. In addition, sampling involving headspace solid phase microextraction (SPME) was used for the identification of bath salts, to determine their volatile organic odor composition to provide greater reliability for detection by law enforcement canines. Gas chromatography chemical ionization mass spectrometry was used to characterize bath salts by sampling its volatile organic composition. This coupled with novel canine training methods utilizing a patent pending universal calibrant will allow for the reliable identification of contraband. In addition to SPME, Fabric phase sorptive extraction (FPSE), a new generation sample preparation technique that possesses over 200 times more sorbent loading surface area than SPME, was developed and will be presented. This highly sensitive microextraction medium allowed the extraction of non-volatile, polar, medium polar, nonpolar organic pollutants, ionic species, organometallic compounds as well as heavy metals. The superior performance and ease of use of FPSE will be discussed for the sampling of environmental water, urine and raw milk for antibiotics hormones, BTEX, pesticides and polycyclic aromatic hydrocarbons. As a result of the high preconcentration factor achieved when using FPSE, no solvent evaporation and sample reconstitution is needed, significantly reducing sample prep as well as minimizing toxic solvent usage.
Ambient ionization sources for mass spectrometry are promising technologies for use in fieldable forensic mass spectrometry. While current ambient methods are suitable for molecular analysis in the field, there has been limited development of ambient ionization methods appropriate for elemental analysis. In forensics, elemental analysis is critical in trace evidence analysis as well as in sourcing the origin of explosive devices. The focus of this talk is on the development of technologies for atomic mass spectrometry and combining those methods with molecular ionization sources. To this end, a microwave plasma torch (MPT) was coupled with a Thermo LTQ XL ion trap mass spectrometer and used to directly analyze the elemental and isotopic composition of a variety of substrates. Materials can simply be placed in the plasma without sample preparation at ambient pressure to yield atomic composition. As little as 10 \[μ\text{g}\] of material was detected using direct MPT sampling. At lower powers, the MPT yielded molecular ions for compounds on the substrate surface. The MPT can be used either for direct elemental or molecular analysis of a solid substrate by simply varying the power. Additionally, the MPT was coupled with laser ablation (LA-MPT-MS) to enhance sensitivity in a technique that is a fieldable alternative to LA-ICP-MS. Current work is focused on coupling the MPT with a portable mass spectrometer (MassTech MT Explorer 50) for fieldable, multimode atomic and molecular analysis.
Isotope ratio mass spectrometry (IRMS) is becoming more user friendly and a more common tool for answering a variety of forensic, ecological, geological, anthropological and environmental questions. In the forensic community, IRMS is already in use in many government forensic laboratories and has passed Daubert standards for admissibility in court on many occasions. This presentation provides two studies involving source attribution using IRMS.

In the first part of the talk, we use two different methods to classify and attribute human hair to subject groups such as body mass index, age and sex. One method uses the absolute abundance of the amino acids in human hair, as determined by derivatization GC-MS. The second approach uses bulk- and amino-acid-specific isotope analysis as input variables for classification. Statistical techniques such as canonical discriminant analysis (CDA) are used to overlook the covariance of amino acid values between individuals caused by dietary factors and instead highlight the selective differences caused by grouping factor(s) such as age, body mass index and sex.

The second part of the talk uses isotope ratios to link blowfly larvae, pupae and adult flies to different food (carrion) sources. Ecologists often use isotope ratio analysis to determine the trophic level of organisms and their primary food sources. However, such analyses are rarely interested in linking adult insects to a specific meat source in a forensic context. In this study, we present a proof of concept study to test the hypothesis that adult blowflies can be linked to specific food sources via their stable isotope ratios.

Keywords: Amino Acids, Chemometrics, Forensics, Isotope Ratio MS
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
The development of microfluidic paper-based analytical devices (µPADs) is described for application to rapid, on-site detection of improvised explosives. Five lane µPADs were designed and printed using wax ink on chromatography paper to create hydrophobic channels. Each channel contains colorimetric reagents capable of reacting with one or more explosive compounds resulting in a specific colorimetric reaction. Two devices were prepared, each capable of performing five simultaneous analyses on a single µPAD. The first µPAD was developed to detect inorganic explosives such as black powder, flash powder, and ammonium nitrate. It detects nitrates, nitrites, chlorates, and perchlorate oxidizers, as well as ammonium. The second µPAD was developed to detect military explosives such as TNT and RDX along with other high explosives like urea nitrate. It also detects organic peroxides such as TATP and hydrogen peroxide. All experiments were performed by dissolving the explosives in deionized water or 50:50 acetone/ H2O as transport solvents with a detection time of around 5 minutes. Detection limits ranged from 0.39 – 19.8 µg of explosive compound. These two customized µPAD devices permit the on-site forensic testing of unknown explosives, thereby supplying law enforcement and first responders with a resource for rapid and simple detection of explosive compounds.

Keywords: Forensic Chemistry, Lab-on-a-Chip/Microfluidics
Application Code: Homeland Security/Forensics
Methodology Code: Microfluidics/Lab-on-a-Chip
The Curiosity rover has been exploring the geology and habitability of Gale crater near Mars’ equator since 2012. Its ChemCam instrument suite provides laser-induced breakdown spectra (LIBS) of rocks and soils up to 7 m from the vehicle and also provides the highest resolution remote images from the rover. The fine-scale LIBS spot size (0.3-0.5 mm) has opened new vistas for Mars geochemistry, with observations at > 4500 locations on > 1000 targets along the rover’s traverse. Each observation consists of multiple laser shots which first remove surface dust, then probe into the surface of the rock. Accurate pointing has facilitated vertical line-scans within the rover’s drill holes as well as chemostratigraphy—analyses across multiple rock layers at an outcrop. New rover software is being tested to robotically select targets based on albedo, size, and shape. The ChemCam spectral database, archived by NASA with nearly 200,000 spectra, represents one of the largest publicly available calibrated LIBS data sets and will be mined by scientists for years to come.

Results include the discovery that Mars soil and dust is hydrated (1.5-3 wt % H2O). The soil consists of multiple components, some of which reflect weathering of local rocks. Igneous float rocks are alkaline and appear to originate in more evolved magmas than those found at previous landing sites, indicating that Mars is more diverse than previously observed. ChemCam provided the first comprehensive window into trace elements like Li, F, Rb, and Sr, and has found strong Mn and Zn enrichments, apparently emplaced by subsurface fluids. As the rover nears Mt. Sharp we expect to discover new insights into the geology and habitability of Mars.

This presentation will also provide details of the SuperCam instrument selected for NASA’s Mars 2020 rover. SuperCam includes the LIBS and imaging capabilities of ChemCam and adds co-boresighted stand-off Raman, time-resolved fluorescence, and infrared reflectance spectroscopies.

**Keywords:** Chemometrics, Environmental Analysis, Infrared and Raman, Robotics

**Application Code:** Other

**Methodology Code:** Portable Instruments
The determination of lowest level amounts of certain elemental or even isotopic species in various types of analytical samples is of highest relevance for a multitude of research fields and applications. In particular, long-lived radioisotopes of natural or anthropogenic origin are of concern not only regarding radiotoxicity in the environment but may also serve as tracers for dedicated geological, astrophysical and increasingly bio-medical studies. Multi-step resonant atomic excitation and ionization using tunable lasers is coupled to well adapted mass spectrometry to provide the essential selectivity in respect to the element under study. Suppression of isobaric contaminations and the high ionization efficiency of the RIMS technique ensures lowest detection limits. Today it is applied e.g. for routine inspection of Pu isotope content and composition in environmental samples with LODs as low as $10^{-5}$ atoms corresponding to activities below mBq. In addition, analytical studies and spectroscopic investigations on $^{41}$Ca, $^{90}$Sr, $^{99}$Tc as well as the minor actinides Np, Am, Cm up to Es are carried out. Revealing the complex atomic structures and deriving ionization potentials serves as prerequisite for further optimizing analytical atom counting. In the cases of $^{90}$Sr and $^{41}$Ca, highest isotopic selectivity is realized by employing the optical isotope shift through narrow bandwidth laser radiation to suppress contributions from the stable isotopes of these ubiquitous elements. Finally the resonance ionization technique is nowadays leading in the field of online production of short-lived exotic radioisotopes at large scale research facilities like CERN in Geneva, Switzerland.

References:

Keywords: Atomic Spectroscopy, Elemental Analysis, Laser, Mass Spectrometry
Application Code: General Interest
Methodology Code: Atomic Spectroscopy/Elemental Analysis
In the past decade there has been rapid growth in the use of low-power plasmas as sources for ambient ionization mass spectrometry. The plasmas differ in their geometries, excitation modes, powers, and support gas composition. Although individual plasmas have been studied with optical spectroscopy, and some comparisons among different plasma types have been reported, it has proven difficult to make direct connections between the fundamental plasma properties and mass spectrometric performance. We will present detailed characterizations of four distinct ambient ionization plasmas based on optical spectroscopic measurements, and will draw connections, where possible, between the plasmas' optical characteristics and their performance as ambient ionization sources for mass spectrometry.

Keywords: Atomic Spectroscopy, Characterization, Mass Spectrometry
Application Code: High-Throughput Chemical Analysis
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The development of knowledge on nanotechnology is having a great impact in analytical chemistry like in mass spectrometric techniques, SERS, high resolution microscopy and gas sensors. The nanometric scale of particle dimension causes peculiar physical features for what concerns their electrical, optical and thermal properties that have been intensively investigated in the last decade.

Starting from these considerations we have recently proposed Nanoparticle Enhanced Laser Induced Breakdown Spectroscopy (NELIBS) in order to enhance the efficiency of the laser-matter interaction and to consequently increase the emission LIBS signal. NELIBS is based on the deposition of a colloidal solution of noble metal NP on the sample surface and virtually does not require any change in the LIBS set-up. As examples in the case of conducting samples, NELIBS allows a signal enhancement up to two orders of magnitude as a consequence of electron field emission during the laser-matter interaction. Instead in the case of transparent media, it is possible to obtain enhanced spectra with respect to conventional LIBS by inducing changes in the optical properties, without damaging or cracking the sample itself.

In this work the fundamental aspects of NELIBS is discussed in correlation with quantitative analysis of different kind of samples ranging from metals and alloys to glasses. Further perspective of nanoparticles applications to LIBS will be discussed as well.

Keywords: Elemental Analysis, Materials Characterization, Nanotechnology, Plasma
Application Code: Materials Science
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Inductively coupled plasma–atomic emission spectrometry (ICP–AES) has become the workhorse for routine multi-element determinations at major to trace levels. However, it can suffer from errors of 30% or more when the matrices of the sample and the calibration standards are not matched. Worse, there is generally no simple way to tell if such interferences are present. It is clearly desirable to have available a method that flags and warns the analyst when an interference exists.

There are three categories of matrix effects (sample-introduction-related, plasma-related, and spectral interferences), and their characteristics and behavior are all different. Recently, we developed a unified interference-flagging method that is effective for all three matrix-effect categories. Moreover, this indicator can be employed in an online fashion during an analysis, is applicable to both conventional lateral-viewing (i.e., side-on) and axial-viewing (i.e., end-on) observation modes, and can be automated.

This simple all-in-one indicator is based on the fact that plasma behavior and excitation conditions vary from one location to another in the ICP. As a result, the relative magnitude and even the direction of the change in emission intensity caused by a matrix are not constant, but are spatial-location dependent. The addition of a new dimension – the spatial direction – to ICP measurements then allows any matrix interference, if present, to be automatically flagged. To elaborate, because the apparent concentration of an analyte in a sample is related to the measured intensity, any change in matrix effects along the spatial profile will cause the determined concentration to be spatially dependent, thus allowing the interference to be detected. In this presentation, the theoretical basis of this matrix-effect indicator and the spatial characteristics of matrix interferences in both ICP observation modes will be discussed, and their effectiveness will be evaluated.
**Session Title**  
Innovative Learning Experiences for Analytical Chemistry and Instrumental Analysis

**Abstract Title**  
IF-AT Scratch-Offs Engage Analytical Students with the Chemical Literature

**Primary Author**  
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**Abstract Text**
Immediate Feedback Assessment Technique (IF-AT) is a multiple choice scratch-off sheet that allows students to learn from their mistakes. Developed by psychology professors Dr. Michael Epstein and Dr. Gary Brosvic, IF-AT is based on psychological principles related to learning and memory. This approach of “answer until correct” using IF-AT scratch-offs has been used in both sophomore level analytical chemistry and junior/senior level instrumental analysis courses at Westminster College since 2007. IF-AT is a low-tech method for facilitating learning during an assessment and has been proven to improve retention of content. At Westminster, IF-AT is used in conjunction with chemical literature reading assignments where students work in small groups on in-class journal article quizzes. They debate each multiple choice possibility and come to a consensus, ultimately celebrating correct answers and critically analyzing incorrect answers before scratching off a different selection. This engaging approach improves students’ understanding of the chemical literature as well as reinforces the analytical content.

**Keywords:**  
Teaching/Education

**Application Code:**  
Other

**Methodology Code:**  
Education/Teaching
Analytical methods can be taught in any setting with a little planning. I have had the opportunity to teach in a very small town in Southern France twice and have brought analytical chemistry into the fold both times. Preparing labs for study aboard courses is very challenging when you have to bring all the materials needed for the entire course. Fortunately companies are making instrumentation that is perfect for this type of adventure.

Ambialet, France is a perfect sight for teaching chemistry, especially environmental chemistry. Surrounded by the Tarn River, the number of water samples to test is infinite. Water quality assessment is easily taught using the Pasco GLX units for data collection. The Pasco GLX units are compact and have many useful probes for testing chemical properties of water. Portable spectrometers and gas chromatography units are also available for analysis and attach easily to student laptops with Pasco software.

Tying the class into Ambialet’s history was easy since mining was a major part of industry in the area. With mining comes the need for metals analysis. Metals analysis is also important for water quality in the old buildings in the area. A compact instrument for metals quantitation is a potentiostat for electrochemical analysis of metals. Two pine potentiostats were utilized to expose students to detection and quantitation of metals. Statistical analysis and literature research rounds out the experience for our chemists and world travelers.

Keywords: Electrochemistry, Environmental
Application Code: Environmental
Methodology Code: Electrochemistry
Abstract Text
The development of microfluidic devices is a forerunning theme in analytical chemistry research. While undergraduate students are now being exposed to concepts of microfluidics and lab-on-a-chip devices in lectures, only a small number of laboratory experiments have been reported in the chemical education literature. To this end, an advanced analytical chemistry laboratory module has been developed. This module has been designed to provide students with an experiential learning opportunity that includes exposure to practical skills development for the cleanroom-free fabrication of microfluidic devices, the opportunity to explore fluid flow in microchannels (through zeta potential, electroosmotic flow velocity, Péclet number, and diffusion coefficient determinations), and the opportunity to develop a lab-on-a-chip device for analysis of a DNA ladder sample that incorporates microchannel electrophoresis and on-line line fluorimetric detection.

The outcomes of work done on the development and implementation of this 4-week laboratory module will be presented. Feedback received from students that have completed this laboratory module and refinements made to improve the module based on this feedback and student observation will also be included.

Keywords: Lab-on-a-Chip/Microfluidics, Laboratory, Teaching/Education
Application Code: Other
Methodology Code: Microfluidics/Lab-on-a-Chip
Innovative Learning Experiences for Analytical Chemistry and Instrumental Analysis

Instrumental Analysis of a Crime

The laboratory portion of the analytical chemistry course can be made more relevant and interesting by incorporating a forensic chemistry project as a culminating activity. A variety of physical evidence – instrumental technique pairings can be wrapped into a crime scenario to form the basis of a multi-week project. Examples of the pairings include the discovery of ignitable liquid residues by GC-MS, identification of bulk drugs by ATR-FTIR, matching of inks from pens and paper by LC with multi-wavelength detection, and characterization of glass shards by atomic spectrophotometry. The author’s decade-long experience with these forensic analytical chemistry laboratories will be described.

Abstract Text

Keywords: Analysis, Forensic Chemistry
Application Code: Laboratory Management
Methodology Code: Education/Teaching
Innovative Learning Experiences for Analytical Chemistry and Instrumental Analysis

A Novel Experience for Undergraduates to Develop Their Own Laboratory Experiment During Their Instrumental Analysis Course

Throughout the semester of the Instrumental Analysis course, the students are introduced and given the opportunity to use an atomic absorption spectrometer (AA), a laser induced breakdown spectroscopy (LIBS) system, a UV/visible spectrometer, a fluorimeter, a Raman spectrometer, a Fourier transform infrared (FTIR) spectrometer, a gas chromatograph/mass spectrometer (GC/MS), and a high pressure liquid chromatograph (HPLC) through various laboratory experiments. These experiments use around nine weeks of the semester. For the remaining six weeks, the students are assigned a project where they develop their own lab using any of the above instrumentation. They are given guidelines to follow. The students meet with me individually to discuss their idea (which does not have to be novel) and to determine if it is feasible. Once they are given the approval to continue with their idea, they have to prepare their own standards, obtain and prep their samples, and work on the method development for analysis. A final aspect of this assignment is that the students are required to write a research paper on their topic; they are to follow specific guidelines which mimic what would be completed for a real journal submission.

There have been numerous projects developed from this assignment over the past two years. Students take the experimental part very seriously and have done some excellent work. Some projects that have been developed include analyzing gasoline samples for ethanol concentration using the GC/MS, analyzing lead in paint using LIBS, calculating the potassium concentration in bananas using the FAA, and determining the naproxen concentration in over-the-counter migraine medicines using HPLC. Overall, this project is a great learning experience for the students and the students generally enjoy it because it is their own idea.

Keywords: Laboratory, Molecular Spectroscopy, Separation Sciences, Spectroscopy
Application Code: General Interest
Methodology Code: Education/Teaching
Innovative Learning Experiences for Analytical Chemistry and Instrumental Analysis

Undergraduate Analytical Chemistry: A Semester-Long Mine Drainage Study Within the Framework of an Analytical Chemistry Lab Course, with Research, Community Service, and Professional Service Opportunities for Students

This presentation will focus on analysis of runoff from the abandoned Marchand coal mine and remediated at the Lowber abandoned mine drainage (AMD) treatment facility in southwest Pennsylvania, and its application to the CHEM 0260 (Introduction to Analytical Chemistry Laboratory) course offered annually at Pitt-Greensburg. This project, begun during the Fall Term 2013 offering of CHEM 0260, is a collaboration with the Sewickley Creek Watershed Association (SCWA) and the Rho Theta Chapter of the Gamma Sigma Epsilon National Chemistry Honor Society. AMD is the most common form of water pollution in southwest Pennsylvania, due to numerous abandoned coal mines that accumulate water with time. The insoluble hydrated iron(III) oxides produced may potentially contaminate streams and render well water unfit for human consumption. The objectives of this project include: 1) determination of selected analytes in AMD within the 15-week framework of the CHEM 0260 course, 2) introducing students to sample collection and preparation, and analytical methodologies for determination of analytes in AMD, 3) reinforcement of the importance of accuracy and precision in all determinations, and 4) exposing students to community and professional service by providing SCWA and Rho Theta with reliable results for analytes in AMD.

Sample collection and preparation, determination of analytes (alkalinity, acidity, pH, sulfate, chloride, total suspended and dissolved solids, iron, aluminum, manganese, and calcium), and the results of determinations performed to date, future plans for this project, and collaboration with the SCWA and Rho Theta, will be presented and discussed.

Keywords: Environmental Analysis, Sampling, Teaching/Education, Water
Application Code: Environmental
Methodology Code: Education/Teaching
Method translation for reversed phase, gradient elution methods is a frequently encountered task but one that requires careful consideration of many variables to maximize the likelihood of success. Failure to properly account for even one key variable can lead to undesirable changes in selectivity or failure of the transferred method. This issue has been discussed extensively in the past but its relevance has reemerged recently by the accelerating trend of replacing existing HPLC methods with new ones relying on UHPLC systems and columns. Additional variables must be adjusted to account for differences between HPLC and UHPLC columns and systems such as when switching from columns made with fully porous to core shell sorbents. In this presentation, the key variables to account for during HPLC to UHPLC method transfer (column porosity, system delay, column optimum flow rate, etc.) will be reviewed and recommendations for successful method transfer will be given. Additionally, a method translation tool that incorporates many of the key variables, thus greatly simplifying method translation, will be shown.

Keywords: Clinical/Toxicology, Environmental Analysis, HPLC, Method Development
Application Code: Other
Methodology Code: Liquid Chromatography
**Session Title**: Successful HPLC Method Development and Method Transfer with Core-Shell and UHPLC Columns

**Abstract Title**: The Expanding Family of Superficially Porous Particles and the Benefits for Easy Method Development and Transfer

**Primary Author**: Xiaoli Wang

**Author**: Agilent Technologies

**Co-Author(s)**: Anne E. Mack, Jason Link, William Long, Wu Chen

**Date**: Thursday, March 12, 2015 - Morning

**Time**: 09:05 AM

**Room**: 274

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**Abstract Text**

Superficially porous particle (SPP) technology has been rapidly adopted by chromatographers in the past several years due to its convincing performance advantage over totally porous particles (TPP). This is evident from the numerous commercial introductions of large number of new SPP products, including more particle sizes (e.g. 1.7um or 4um), more pore sizes (e.g. larges pore for proteins and peptides) and more phase chemistries (e.g. almost all phases on totally porous particles are available on superficially porous particles).

The wide variety of phases available on SPP greatly facilitates method development. Using the Hydrophobic Subtraction Model, we will show that different phase chemistries, such as polar-embedded phase, PFP phase and cyano phase, give different selectivities compared to C18 phase. In addition, we will discuss an innovative hybridized superficially porous particle that is designed to resist degradation in high pH mobile phases. It is well known that mobile phase pH can substantially change selectivity for ionisable compounds but operation in high pH requires chemically stable columns. By using this new high pH stable SPP column, one can run separations in low, neutral and high pHs as a way to gain different selectivity. We will show examples of the combination of different phases on silica-based SPP and the hybridized SPP in a method development system to help find the optimum method to achieve good resolution in a short amount of time, taking advantage of the speed offered by the SPP technology.

We will also discuss method transfer from totally porous particles to superficially porous particles. The difference in particle porosity due to different particle morphology will be considered in method transfer to ensure the same selectivity is obtained. Practical examples in both isocratic and gradient mode will be shown.

**Keywords**: HPLC, HPLC Columns, Method Development, Pharmaceutical

**Application Code**: Pharmaceutical

**Methodology Code**: Liquid Chromatography
Successful HPLC Method Development and Method Transfer with Core-Shell and UHPLC Columns

The Critical Choice of Stationary Phase Chemistry during HPLC Method Development

Many of the problems encountered executing HPLC methods are a result of decisions made during the early method development process. The objective of this effort is to discuss the critical variable of stationary phase chemistry. In practice, many analysts will reach for their favorite C18 upon commencement of method development; however alkyl (Cn) phases are often not the most appropriate tool for a given set of separation. When retention or resolution is not readily achieved, analysts will often resort to the addition of ion-pair reagents or other complicated mobile phase preparations to force them to work. This common practice often results in the development of complex methods that suffer from lack of transferability, robustness and ruggedness.

There are many choices for alternative stationary phase chemistries that render the phase decision difficult. In this work we discuss two stationary phase classes, embedded polar group (EPG) and aromatic that are highly complementary to alkyl phases from a fundamental molecular interaction point of view. An understanding of the contrasting interactions that these different classes of stationary phase chemistries provide leads to more effective decisions during the process. This critical information promises to facilitate method development and generate simpler, more reproducible separations.

Keywords: HPLC, Method Development
Application Code: General Interest
Methodology Code: Liquid Chromatography
Several advantages have been demonstrated through the use of fast and high efficiency HPLC column technologies. Column technologies such as those of sub-2 micron and superficially porous particles have been evaluated extensively and display the potential to provide benefits across various industrial applications. However, as alluring it may be to incorporate these technologies, some considerations should be taken into account in a pharmaceutical setting. One major consideration is the capabilities of the laboratory that will eventually need to run the method. In some instances a laboratory may not have the proper instrumentation and/or training to incorporate these column technologies which can lead to undesired consequences during method transfer. Furthermore, options to proactively avoid this issue can be complicated by regulatory bodies of various countries requiring testing in laboratories on their soil.

In this presentation we explore the scalability of HPLC methods across various column technologies for a number of pharmaceuticals. Successful scaling is demonstrated by changing column format while maintaining specificity. This approach provides laboratories with the ability to tailor a method based on needs and capabilities with minimum to no additional method development efforts.

Keywords: Chromatography, HPLC, Liquid Chromatography, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Successful HPLC Method Development and Method Transfer with Core-Shell and UHPLC Columns

HPLC Method Development and Transfer with Core-Shell Columns for Agricultural Applications

High performance liquid chromatography (HPLC) is the predominant analytical technique in pharmaceutical, agricultural and food industries. Modern quality control (QC) laboratories rely heavily on HPLC analysis for product release and process monitoring. More reliable, efficient and cost-effective analytical methods are desired by the industries. Recent advancement in LC column technologies led to significant improvement in separation efficiency and method reliability. Column packed with core-shell particles allowed faster and more efficiently separation to be carried out on conventional instruments and is a lower pressure alternative to columns packed with sub-2-µm particles. Albeit the advantages, there are practical considerations to be taken in order to take full advantage of these new technologies. In this presentation, a few applications in the separation of natural product and small molecule agricultural products will be given and benefits and limitations of these advanced LC column technologies will be discussed. Systematic method development and method transfer with core-shell columns will also be discussed.

Keywords: Agricultural, Chemical, Chromatography, HPLC Columns
Application Code: Agriculture
Methodology Code: Liquid Chromatography
To support in vitro human skin penetration studies of topically applied anti-wrinkle peptides, a rapid hydrophilic interaction liquid chromatography with tandem mass spectrometry (HILIC-MS/MS) method for the simultaneous quantitation of two peptides, Ac-EEMQRR-amide and H[sub]2[/sub]N-EEMQRR-amide, was evaluated and successfully applied to the analysis of various samples including emulsions, receptor fluids, cotton-tipped applicators, and human skin layers such as stratum corneum tape strips, epidermis, and dermis. Stable isotopically labeled peptides, corresponding to native Ac-EEMQRR-amide and H[sub]2[/sub]N-EEMQRR-amide, were used as internal standards to correct for loss of recovery and matrix effects. Electrospray ionization (ESI) was used in the positive mode. Depending on the sample matrix, this analytical procedure involved extracting samples with 0.1:0.1:85:15 trifluoroacetic acid:formic acid:acetonitrile:water (v:v) or with 0.05:90:10 ammonium hydroxide:acetonitrile:water, followed by analysis using HILIC-MS/MS. For receptor fluids, epidermis, and dermis samples, it was necessary to first separate the two peptides from the sample matrix using HILIC-solid phase extraction (SPE) to minimize the serious ion suppression prior to HILIC-MS/MS analysis. The recoveries for Ac-EEMQRR-amide and H[sub]2[/sub]N-EEMQRR-amide ranged from 89% to 101% and 80% to 100 %, respectively, at three spiking levels for the stratum corneum, epidermis, and dermis samples. The amount of Ac-EEMQRR-amide ranged from 249 ng/cm[sub]2[/sub] detected in the first layer of the stratum corneum to 5.3 ng/cm[sub]2[/sub] found in the thirteenth layer of the stratum corneum. The amount of Ac-EEMQRR-amide found in the epidermal layer was 23 ng/cm[sub]2[/sub]. Neither of these peptides was detected in the dermis or receptor fluid samples. H[sub]2[/sub]N-EEMQRR-amide was not detected in any samples.
Black cohosh (Actaea racemosa L.) has become increasingly popular as a dietary supplement in the United States for the treatment of symptoms related to menopause. However, it has been known to be adulterated with the Asian Actaea dahurica (Turcz. ex Fisch. & C.A.Mey.) Franch. species (syn. Cimicifuga dahurica (Turcz.) Maxim). It is important to develop a method for the identification and differentiation of American black cohosh from other closely related species. However, the existing methods for identification and differentiation of black cohosh are usually long and laborious. Mass spectrometric fingerprints were obtained in the negative ion mode using flow injection mass spectrometry (FIMS) for 77 black cohosh samples and their related species. The analysis time for each sample was less than 2 min. All data acquired were processed using principal component analysis (PCA).

Keywords: Bioinformatics, Characterization, Food Identification, Liquid Chromatography/Mass Spectroscopy
Application Code: Food Identification
Methodology Code: Mass Spectrometry
Allergens are well known to affect between 1-3% of the population. Affected consumers have to avoid the offending proteins at all cost. If a person is milk protein allergic, it is obvious that milk should not be consumed. But how about processed products? If you are soy allergic, and a product contains vegetable proteins, does it contain soy? In the worst case scenario, allergens end in a product through cross contamination or carry – over. In those cases, the offending allergens will not be mentioned in the ingredient list, and the only option is to analyse. But to what levels should be analysed? This question is equally important for food manufacturers who need to know if they production lines are sufficiently clean, as it is to consumers, who need to know if the allergen is potentially present. In many parts of the western world Risk Assessment Agencies set limits for dangerous substances. For allergenic consumers, certain proteins are dangerous. So far, none of the agencies has set thresholds for allergens, above which products should be labelled, even if the allergen is not part of the recipe. Clinical research has generated plenty of data and scientists seem to agree that thresholds for most allergens should be in the lower ppm (mg/kg) range. But without legal framework those values may not hold if challenged in court. But even if threshold values had been set: would food industry and consumer be better off? That is questionable, since one of the main impact factors has not really been addressed: the analysis. At present, especially with highly processed foods, analytical methods can give strongly varying results. Those methods range from PCR and immunological methods (lateral flow device, sandwich ELISA, competitive ELISA) to mass spectrometry. And even within the same group of methods, (e.g. immunological assays), results can vary significantly. So how can confidence for food manufacturers and consumers be achieved?
Ambient mass spectrometry (AMS) has been used to perform analyses under ambient conditions without sample pretreatment. The ESI-based AMS is useful for characterizing polar and large compounds, while APCI-based AMS is useful for characterizing small, less polar or even nonpolar compounds. A combination of both ionization methods will extend the detectable mass range as well as polarity. In this study, we combined a thermal desorption laser sampling unit with a mass spectrometer equipped with a dual ambient ESI+APCI source so both polar and nonpolar polymeric fragments released from food packing polymeric materials were rapidly characterized.

The ion source having the functions of ESI and plasma APCI was constructed by inserting a fused silica capillary into a stainless steel tube that was enclosed in a glass tube. An AC high voltage was applied to the ring electrode attached to the glass tube to discharge a stream of N2 gas flowing between the stainless steel and glass tube. A DC high voltage was applied to the ESI solution to generate an ESI plume at the capillary tip. A piece of food packaging material was set on a titanium foil and was pyrolyzed by laser irradiation. The desorbed neutrals were post-ionized via subsequent reactions with the reactive species in ESI and plasma APCI plumes. In addition, by controlling the high DC and AC voltages applied on the dual ionization source, the ionization can be operated in ESI-only, APCI-only and ESI+APCI mode, respectively. This technique was used to rapidly characterize the chemical compounds in various food packing materials which were made by different types of polymers including polyethylene (PE), polypropylene (PP), polystyrene (PS), poly(methyl methacrylate) (PMMA), polylactic acid (PLA), polycarbonate (PC), synthetic rubbers, etc. Besides, principal components analyses (PCA) was used to rapidly classify the polymeric materials based on the results from laser desorption-ESI+APCI/MS.

**Keywords:** Electrospray, Laser Desorption, Mass Spectrometry, Polymers & Plastics  
**Application Code:** Food Safety  
**Methodology Code:** Mass Spectrometry
The analysis of very polar compounds in food has been considered to be one of the most challenging topics. The polar compounds are usually eluted in the void volume when separated with reversed-phase LC (RPLC). However, they may be the important target components in food analysis. A novel stationary phase was introduced in this study, and its advanced applications were demonstrated for food safety.

Abstract Text

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Keywords: Food Safety, HPLC Columns, Liquid Chromatography/Mass Spectroscopy

Application Code: Food Safety

Methodology Code: Liquid Chromatography/Mass Spectrometry
Dietary copper deficiency is associated with a variety of manifestations of the metabolic syndrome, including hyperlipidemia and fatty liver. In order to investigate whether copper deficiency plays a role in fructose-induced fatty liver, male weanling Sprague-Dawley rats (35–45 g) were housed in stainless steel cages in a temperature and humidity controlled room with a 12:12 h light–dark cycle. All rats were allocated into four groups. Each group of rats was fed either an adequate copper or marginally copper deficient diet. At the same time, distilled water or distilled water containing 30% fructose (w/v) was given ad lib for 4 weeks. Fructose enriched drinking water was changed twice a week. At the end of the experiment, all the animals were killed under anesthesia with pentobarbital (50mg/kg I.P. injection). Feces were collected and snap-frozen with liquid nitrogen for metabolomics study. Metabolites were extracted using a solvent mixture of methanol and water. The extracted metabolites were derivatized using N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA). The derivatized samples were analyzed on both LECO Pegasus 4D GC×GC-TOF MS and GC-HRT-MS instruments, respectively. Experimental data were processed by ChromaTOF software for peak selection and compound identification, followed by MetPP software for retention index matching and metabolite quantification. Preliminary analysis shows a significant metabolite profile difference between sample groups. Several analytes not confidently identified in the GCxGC-TOF MS experiment have been assigned identities based on the accurate mass analysis in GC-HRT-MS data. The fecal metabolites profile reveals novel mechanism underlying marginal copper high fructose diet-induced NAFLD.

Keywords: Bioinformatics, Gas Chromatography/Mass Spectrometry, Metabolomics, Metabonomics

Application Code: Food Safety

Methodology Code: Other
Mass spectrometry (MS) instruments have significantly advanced in recent years, providing superior sensitivity, selectivity and ruggedness. Therefore, modern MS detectors can be used not only as a detection tool for chromatography, but also as a stand-alone detection / identification tool, thus allowing to eliminate time consuming chromatographic separation. The objective of this study was to develop and evaluate a novel screening method for selected pesticides and mycotoxins in cereal-based food samples using flow injection tandem mass spectrometry (FI-MS/MS). To ensure reliable MS identification in absence of retention time (tR) in FI-MS/MS analysis, additional MS parameters, such as enhanced product ion and multi-stage MS (MSn) were investigated. Prepared extracts of wheat, rye, barley and soy were ten-fold diluted and fortified at 10, 50 and 500 ng/g of the contaminants. The identification was performed using LC-MS/MS QTrap (API 5500, Applied Biosystems) with electrospray ionization in positive mode. Ion suppression, interferences and selectivity were evaluated. The method detection limits (MDL) were 1 - 4 ng/g for aflatoxin B1, fumonisin B1 and B2, ochratoxin A, T2 and HT2 toxins; and 8-12 and 5-25 ng/g for zearalenone and deoxynivalenol, respectively. For pesticides, the MDLs were at or below 10 ng/g. The results suggest the method is applicable for fast screening of selected pesticides and mycotoxins at or below the established maximum residue levels in grains/cereals.

Dr. Sapozhnikova acknowledges the receipt of a fellowship from the OECD Co-operative Research Programme: Biological Resource Management for Sustainable Agricultural Systems in 2014.

Keywords: Flow Injection Analysis, Food Contaminants, Food Safety, Mass Spectrometry
Highly-developed downstream processes require a strict quality control of the generated synthesis gas. Being poisons to the used catalysts, the determination of sulfur compounds is absolutely crucial. Despite fulfilling these demands, sulfur selective detectors, like the Sulfur Chemiluminescence Detector, can be difficult to operate.

This work deals with a feasibility study of an alternative analytical system consisting of a Compact-GC equipped with a Plasma Detector. The work is focused on the determination of the detection limits of the different components, repeatability and general performance of the detector.
Tiger Optics has developed the Prismatic 2, a new generation of Continuous-Wave Cavity Ring-Down spectrometer that transcends the bandwidth limitation of high-reflectivity mirrors by employing Brewster prisms, which exploit total internal reflection, to form a broadband, high-finesse cavity. Integration of multiple diode lasers, combined with such a cavity, allows real-time detection of multiple analytes with very high sensitivity.

The Prismatic 2 analyzer is best suited to serve applications requiring detection of multiple analytes in a single gas stream. Multi-species capability is required in a wide range of applications such as quality control of high-purity gases, hydrogen analysis for fuel cells, and emission monitoring. In fuel cell applications, it is crucial to monitor contaminations in the hydrogen gas because their efficiency and lifetime is dictated by the purity of the hydrogen. This requires simultaneous monitoring of critical impurities, such as CO, CO2, CH4, H2O and others in bulk hydrogen. The Prismatic 2 offers simultaneous detection of these impurities and other trace level contaminants, including NH3, HCN, H2S, and AsH3, as a real-time monitoring system. Therefore, it also provides numerous opportunities for multi-species trace gas detection beyond fuel cell applications. Gas companies typically monitor gas quality using a gas chromatograph and several single-species instruments; the ability to replace these with one instrument significantly simplifies the process and reduces cost, and is therefore affords significant savings, while improving yield and throughput.

The presented data illustrates simultaneous detection of CO2, CO, H2O and CH4 in hydrogen, achieving detection limits of 150, 100, 10 and 1 parts-per-billion (ppb), respectively, and demonstrates fast speed of response, excellent linearity and repeatability over a wide range of analyte concentrations.

Abstract Text
For many reactive gases adsorption and reactions are limiting factors in gravimetric preparation of stable reference gas standards at trace levels. The influence of adsorption of low concentrations of ammonia on cylinder surfaces has been investigated at VSL. This resulted in the development of certified reference gas mixtures at few ppm levels with reduced uncertainty. In order to support the measurement of formaldehyde levels in outdoor and indoor air, research has been conducted to assess the reliability of formaldehyde gas standards in cylinders. The study aimed in particular at evaluating the stability of formaldehyde at few ppm levels. The presentation will provide the results of this study.

Keywords: Air, Reference Material, Specialty Gas Analysis
Application Code: Environmental
Methodology Code: Molecular Spectroscopy
Phosphine is used as a precursor for the deposition of Group III-V compound semiconductors, and as a dopant in the silicon semiconductor manufacturing process. It is used in large amounts for the manufacture of LED and photovoltaic products. In the case of III-V materials such as InP, absence of other hydride dopant impurities such as moisture, silane, germane or hydrogen sulfide is critical as they can have a profound effect on the performance of the final device. In particular, incorporated sulfur from hydrogen sulfide and carbonyl sulfide may affect electrical properties of the device such as carrier concentration and electron mobility. Dopant impurities may also impact the electroluminescence properties of LED products. Determination of these compounds at ppb levels and below is therefore very important. This presentation reviews the analytical technology available using gas chromatography, including column choices and detectors. One of the most challenging measurements has been the determination of hydrogen sulfide due to the difficulty in detection at trace levels as well as its chromatographic behavior with respect to that of phosphine.

Keywords: GC-AED, Plasma Emission (ICP/MIP/DCP/etc.), Semiconductor, Specialty Gas Analysis
Application Code: Materials Science
Methodology Code: Gas Chromatography/Mass Spectrometry
Specialty gas is a somewhat vague term often confused with industrial or bulk gas. While specialty gases, or gas mixtures, are used in industry they differ in the characteristics of high purity and/or complexity and certified composition. A novel vacuum ultraviolet (VUV) absorption spectroscopy technique has recently been introduced that provides unique capabilities for gas phase analysis. The instrument can be used as a gas chromatographic detector or for direct injection gas phase spectroscopy. The instrument rapidly collects continuous absorption spectra from 120nm – 240nm. Most gas phase molecules have very strong and unique absorption cross sections in the VUV, which enable sensitive and selective analysis. Results of the analysis of different specialty gases are presented. The versatility of the VUV detector software is demonstrated using spectral filters to overcome matrix effects, and spectral deconvolution for co-eluting components. Applications such as TO-15, natural gas, permanent gas analysis and the presence of H2S in phosphine will be discussed.
In this paper we will discuss progress on the development of HEMS, a technology used to enrich impurities in hydrogen for analytical applications. The technology consists of a pre-concentrator, coupled with a mass spectrometer-based analytical unit used to detect impurities. By increasing the concentration of impurities, this technology has the potential of reducing the cost of trace analysis in hydrogen for a variety of applications from fuel cells to semiconductors.

In this paper we will focus on HEMS-M, an analyzer designed to verify compliance with hydrogen purity standards SAE-J2719 and ISO 14687. The instrument is designed to be deployed at hydrogen filling stations for real time operation and thus allowing interruption of dispensing hydrogen in the event that the limits on the impurities specified by the standard are exceeded. We will report on progress on the analytical figures of merit for the analytes tested.

The core of the technology is a palladium-alloy micro-channel membrane. The membrane acts as a pre-concentrator by selectively permeating hydrogen, thus enabling an increase of the impurities present at trace levels. The alloy composition, temperature of operation and coating of the membrane are designed to reduce the reactivity of the trace species to be analyzed. Successful enrichment of ammonia in a hydrogen gas stream has been demonstrated. The analytical requirements will be discussed, including the wide dynamic range for the different species.

Keywords: Energy, Fuels\Energy\Petrochemical, Mass Spectrometry, Specialty Gas Analysis
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Mass Spectrometry
Advances in Process Analytical Chemistry


Over the last years, on line analysis has become currently used for the instrumentation of industrial plants (process monitor and control) as well as the equipment of research and development pilot plants (chemical, petrochemical, green chemistry, …). In the last years, new technologies were put in the market like NeSSITM and miniaturized systems which enable new on line analysis concepts. For industrial applications, numerous benefits of these new concepts can be foreseen: reduce greatly the response time, increase the safety level of analysers (reducing transport and volume of samples), decrease the detection limits (no sampling, no contamination), open the way for new optimisation applications in order to perform new measures at low investment cost, define new standards of components, reduce the cost of maintenance with reduction of spares, increase flexibility of analytical applications with easy addition or replacement of micro analysers using the ISA SP 76 standard. Trends in R&D is toward downscaling and process intensification (micro reactors, string pellet reactors), which put new constraints on analytical systems. Downscaling results in less product available and requires the development of new analytical tools and strategies: bring the analysis directly on the process streams. To implement these tools in or as close as possible of the reactor, they must become smaller with no loss of performance. New projects from IDEEL (new French company) aim at fostering innovation in the field of industrial analysis by catalyzing the development of adapted in situ tools. It gathers partners from R&D (academic centers, instrumentalists) to the market (industrialists).

This paper will present some major actual and future trends and needs for on line micro analytical instruments and micro captors for R&D (process intensification) and industrial plants. This paper will be illustrated with different examples of sensors and analyzers: μdensimeter, μGC, μNIR, μRaman.

Keywords: Analysis, GC, Process Analytical Chemistry, Raman
Application Code: Process Analytical Chemistry
Methodology Code: Process Analytical Techniques
A Secure and Easy to Use UPLC System for the Analysis of Samples Close to a Manufacturing Process

Many customers experience serious delays to their manufacturing work flow when waiting for analytical results. Such at risk work flows may be found in various industries such as pharmaceutical, food and commodity manufacturing. An analytical solution for this problem is to provide a secure and easy to use UPLC system for implementation at the manufacturing location.

A novel instrument has been introduced that provides access to the speed, sensitivity and resolution of UPLC technology. This instrument has been designed with security measures to track chain-of-custody compliance ensuring controlled access to the instrument, the samples being analyzed and the results generated. The bar code initiated analysis permits all levels of personnel expertise to run samples and to obtain quantitative results. The instrumentation also provides for multiple levels of dilution ensuring a workflow that decreases or even eliminates the errors in creation of multi-point calibration curves and sample preparation.

Several pertinent product samples, which may be subject to regulatory audits, will be used to illustrate the use of this instrument.

Keywords: Chromatography, Natural Products, Process Monitoring, Sample Handling/Automation
Application Code: Process Analytical Chemistry
Methodology Code: Process Analytical Techniques
Analysis can be found everywhere within Air Liquide. Increasingly, industrial applications require analytical systems which have a higher degree of accuracy at lower levels with an increasing array of components within the sample. These analyses must be conducted in shorter time frames and with less operator intervention.

This presentation will describe the application development for trace hydrocarbon, CO2, N2O analysis in oxygen streams with the unique Very High Resolution Tunable Laser source technology. Detection system developed by ONERA (the french aerospace lab) and Blue Industry and Science.

The developed laser source is tunable over a wide range of the infrared spectrum (between 2300 and 3100 cm\(^{-1}\) in the current version) with a very high resolution (0.01 cm\(^{-1}\)). With a single laser source, the VHR- TL technology works like hundreds of diodes connected in parallel, which can be activated on demand to fit the application needs. This technology yields performances similar to those of TDLAS, with real multi-gas possibilities (more than 300 gases are measurable with detection limits between 1 and 100 ppb).

**Keywords:** Photoacoustic, Process Monitoring, Spectroscopy, Trace Analysis

**Application Code:** Process Analytical Chemistry

**Methodology Code:** Process Analytical Techniques
X-ray Fluorescence (XRF) is a widely accepted technique for elemental analysis of a large variety of materials, including solids, liquids, powders and thin films. Traditionally two techniques exist: energy dispersive and wavelength dispersive XRF, both offering distinct advantages for particular applications, elements and concentration ranges. In this study, recent technical advancements in both energy and wavelength dispersive XRF will be discussed in detail. Various application examples will be shown for which these advancements result in significantly improved analytical capabilities.

Keywords: Materials Science, Process Control, Process Monitoring, X-ray Fluorescence
Application Code: Process Analytical Chemistry
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The Laser Induced Breakdown Spectroscopy (LIBS) is based on the spectrum of small plasma induced by irradiation of high peak power laser applicable to gas, aerosol and solid-phase samples. This method can also detect almost all elements in the sample. A key advantage is that pretreatment is not required as the laser is focused on the sample surface and consequently induces the plasma. These characteristics provide a great advantage for onsite real time analysis in an industrial environment. Our objective is to apply this method for quality control of trace metal particles in liquefied electronic specialty gases.

Initial experiments were conducted with an aqueous sample containing ppm$[\text{w}]$ Zn, Mn, Fe and Cr nano particles. The experiments were conducted using a home-built LIBS with Nd:YAG laser. The laser beam is focused in the middle of the sample solution in a quartz cell and the optical emission light is observed by spectrograph equipped with an intensified charge coupled device as the detector. The probability of the breakdown was quite different for each metal. Zn and Cr were relatively easy to generate the breakdown but Mn and Fe were not. Furthermore, the spectra of constituent metal atoms were observed, but the appearance of the atomic spectra is unstable with a large shot-to-shot difference. The equipment and method evaluation will be discussed of this technique along with the stability and detection limits in static conditions.

Subsequently evaluation of metals in a liquefied gas in a pressure resistant gas cell with a quartz window will be also discussed.
Cell-based assays are used to obtain biochemical information while avoiding the inherent complications of in vivo systems. Though many advances to classic in vitro methods have been made, analysis is still primarily limited to either fast detection of electrochemically active events or time-delayed measurements of non-electroactive species with poor temporal resolution. There remains an analytical challenge in monitoring fast dynamics of non-electroactive molecules in vitro. We have previously developed an alternative in vitro sampling platform by culturing astrocyte cells directly onto a microdialysis probe coupled with an online high-speed capillary electrophoresis (CE) instrument. Dynamic changes in the relative abundance of analytes have been monitored with 20 second temporal resolution. To the best of our knowledge, release events of non-electroactive compounds in culture have not previously been observed on this time scale. To better understand the technical variables of this technique, we have been characterizing how variables such as temperature, size of environment, stirring, and strength of stimulation influence our ability to make these fast measurements. Efforts are also currently being made to couple the in vitro-microdialysis probe to a microfluidic chip capable of performing micro-free flow electrophoresis. Preliminary data suggests that amino acid transmitters such as glutamate and aspartate will be able to be monitored continuously at basal levels and in response to stimulation, allowing us to see fast-dynamics previously lost in temporal resolution.

Keywords: Amino Acids, Capillary Electrophoresis, Neurochemistry, Sampling
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
It is of great importance to understand biochemical systems’ behavior toward environmental perturbations during the development of living organisms. Nevertheless, obtaining high-resolution spatiotemporal information during embryonic development is almost impossible with conventional biotechniques. A microfluidic system capable of developing a Drosophila embryo’s anterior and posterior halves controlled at different temperature was established to study the embryo’s reaction under environmental perturbations. The microfluidic chip (60 mm × 60 mm × 5 mm) consists of a polymethylmethacrylate substrate and a polydimethylsiloxane (PDMS) cover patterned with a “Y” channel network (800 micrometer-wide and 400 micrometer-deep). The PDMS cover was bonded to the substrate with the embryo located right in the channel intersection. Temperature gradients (25°C-16°C, 25°C-18°C, 25°C-20°C) around the embryo were created by introducing two fluids with different temperature through the ‘Y’ side channels. The fluid temperature was measured by a superfine wire thermocouple (125 micrometer, o.d.). The microfluidic device was coupled to a confocal microscopy for real-time image acquisition. Histone-eGFP embryos were developed in the platform and monitored in situ. It was found that the warmer half of the embryo developed faster than the cool half at the beginning, but no difference was observed at the end of each cell cycle. The larger the temperature gradient, the more obvious difference for the development rate, but always within one cell cycle. It implies that the two halves of the embryo communicated during the development and there might be a check point in each cell cycle for the 14 cycles of Drosophila embryonic development.

Figure 1. Development rate difference between the two halves of an embryo exposed to a temperature gradient of 25°C-16°C. (a) Before cleavage stage. (b,c,d) during one cell cycle, (e,f,g) during the next cell cycle. The green fluorescent dots are histone-eGFP labeled nucleus.
Adenosine is an important biomolecule involved in energy regulation in the brain during conditions such as traumatic brain injury (TBI) and stroke. Inosine and hypoxanthine, two metabolites of adenosine and guanosine, another purine nucleoside in the brain, have also been recognized as markers of cerebral ischemia. Under pathological conditions such as TBI and stroke, the extracellular concentrations of adenosine, guanosine, inosine and hypoxanthine in the brain are known to be increased. Simultaneous and continuous monitoring of these biomarkers is extremely important to better understand neurological disorders and also for evaluating potential drug candidates for treatment of TBI and stroke. However, clinical instrumentation for simultaneous online monitoring of these biomarkers is currently unavailable. Microdialysis coupled with microchip electrophoresis with amperometric detection is a powerful technique that can be used for fast and reliable monitoring of biological samples. Microchips electrophoresis with amperometric detection was successfully employed for the separation and detection of all four biomarkers. The chip was fabricated from PDMS and had a 5 cm long separation channel and a carbon fiber working electrode. The run buffer consisted of boric acid at pH 10.0 with 10% (v/v) dimethylsulfoxide and field strength of 222V/cm was applied. Under these conditions, all four compounds could be separated in less than 80s. Ultimately, this method will be coupled on-line to microdialysis sampling to monitor these biomarkers in extracellular fluid of the rat brain during ischemial reperfusion and TBI.

Keywords: Analysis, Electrochemistry, Lab-on-a-Chip/Microfluidics, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Microfluidics/Lab-on-a-Chip
Renal transplantation is the gold standard treatment for end-stage renal failure (ESRF), a disease affecting approximately 870,000 Americans today. Some 400,000 Americans require regular intensive dialysis therapy, travelling many miles to their regional dialysis centre up to 3 times per week. On top of this, they depend on multiple drugs and severely restricted lifestyles just to stay alive.

Approximately 170,000 Americans are living with renal transplants. Not only does a successful transplant restore quality of life and return people to work, but each year of transplant function can lead to individual cost savings of US$50,000 or more.

There are of course some big socio-political and research issues surrounding renal transplantation including organ availability, patient matching and immunosuppression, but one area increasingly recognised as vital to the long-term function of a transplant is the pre-implantation care of the organ.

As yet there is no established methodology for successfully resuscitating and maintaining an organ during the time it takes to travel from its deceased donor to its living recipient, often many miles and hours away.

We have developed a continuous creatinine clearance monitoring system and demonstrate its efficacy in isolated perfused porcine kidneys. Creatinine clearance is a globally accepted clinical standard measure of renal function and reflects organ injury and dysfunction.

We believe this live functional data will change the face of surgical risk stratification and lead to improved patient outcomes as well as cross-fertilising other areas of renal transplantation research.
## Microfluidic Biosensors for the Analysis of Circulating Leukemic Cells: Detection of Minimum Residual Disease for Acute Myeloid Leukemia as a Case Example

Acute myeloid leukemia (AML) is a rapidly dividing tumor that can progress from a state with no morphologic evidence of disease to florid hematological relapse in weeks. Minimum residual disease (MRD) analysis is critical to overall survival. Multi-parameter flow cytometry (MFC) is the most common method of detecting AML MRD but has a poor LOD (1 target cell in 10^4 background cells) and so requires a bone marrow aspirate drawn only once every six months due to patient discomfort. In contrast, we present an integrated microfluidic system to isolate low abundant leukemic blasts from 50-500 µL of peripheral blood. Three microfluidic devices isolate highly pure fractions of three blast subpopulations by positive-affinity selection against CD33, CD34, and CD117 antigens, which together sample 80-90% of all patients. We have previously demonstrated markedly low limits-of-detection (1/10^9) and purities >90% for this technology, which consists of a thermoplastic microfluidic possessing antibody decorated high-aspect ratio curvilinear channels (25 x 150 µm). All subpopulations are immunostained on-chip against a panel of antibodies then sequentially released off-chip by enzymatically cleaving novel oligonucleotide linkers. The released cells are routed to a spectral-MFC system for immunophenotyping, which has an optical train consisting of only laser excitation, a spectrograph, and a CCD camera operated in a time-delayed integration (TDI) mode. In total, the system provides the same phenotypic information as conventional MFC but offers a sensitivity improvement of five orders of magnitude and enables MRD detection using peripheral blood instead of bone marrow aspirates, thereby permitting more frequent diagnostics.

### Keywords:
- Biosensors
- Immobilization
- Lab-on-a-Chip/Microfluidics
- Medical

### Application Code:
- Biomedical

### Methodology Code:
- Microfluidics/Lab-on-a-Chip
Transfusion of blood components to patient recipients has become a daily healthcare activity. Recently, our group has shown that the high concentration of glucose in currently approved blood storage solutions is having adverse effects on the stored red blood cells (RBCs). Investigating all of the storage solutions currently approved by the FDA would be time consuming and require numerous donations of blood. We have developed a 3D-printed microfluidic device that has 12 channels, is designed to fit into a commercial plate reader for high throughput optical measurement, and was re-used for months throughout the study. Next, we modified and maintained the glucose level of 3 popular hyperglycemic storage solutions (AS-1, AS-3, and AS-5) at 5.5 mM, the normal level of healthy humans. Flow-induced ATP released from RBCs stored in standard storage solutions, which are hyperglycemic, and normoglycemic versions of each solution, was determined with the luciferin/luciferase chemiluminescence assay. For example, on the first day of storage, RBCs stored in AS-1 released significantly less ATP (126.7 +/- 2.2 nM) in comparison with RBCs stored in a normoglycemic version of AS-1 (250.9 +/- 14.6 nM) (p < 0.05, n = 4 humans). This trend was continued to day 35 of storage where RBCs stored in AS-1 released 64.8 +/- 8.3 nM ATP, significantly lower than RBCs stored in a normoglycemic version of AS-1 (172.8 +/- 10.1 nM) (p < 0.05, n = 4 humans). The ruggedness of the 3D-printed devices is enabling us to study month-long processes with minimal variability and low-volume sample consumption.

Keywords: Biological Samples, Biomedical, Biotechnology, Lab-on-a-Chip/Microfluidics
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Exposomics is a relatively new recognized branch of science with an environmental human health focus based on realization that non-communicable diseases are caused by toxins in the environment, resulting in epigenetic changes and metabolic alterations in the human body. In a recent four-year study, over twenty unique personal chemistry indicators that are statistically significant at the 95% Confidence Interval have been determined in children with autism as compared to control children. The study was a clinical research trial of children with Autism compared to control children who were age, sex and socioeconomically matched within a one hour radius of Pittsburgh, Pennsylvania, USA. The experiments required over 300,000 individual biometric measurements of organic toxins, metals and elements, and two unique biomarker and epigenetic assays. New Direct Isotope Dilution and Speciated Isotope Dilution methods (D-IDMS and D-SIDMS) adopted by the United States Environmental Protection Agency (EPA) were optimized and applied. These methods are known as the EPA Method 6800, Update V, approved in 2013. The experimental protocol was designed with many goals and specific aims, including improvements or creation of advanced measurements to assist in differentiating the chemistry of the children as early as possible. This chemical differentiation may eventually lead to risk assessment and diagnosis of autism before symptoms and brain damage appear.

**Keywords:** Biomedical, Environmental, Forensic Chemistry, Gas Chromatography/Mass Spectrometry

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
The NexION 350 ICP-MS opens up a whole new world of efficiency and opportunity, allowing you to measure more in less time. PerkinElmer has now coupled our market leading NexION with a number of tailored Informatics solutions enabling simple data integration with advanced visualization and decision support. In this presentation real world examples of these technologies will be highlighted.

Abstract Text

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Keywords: Biopharmaceutical, Data Analysis, Data Mining, Informatics

Application Code: High-Throughput Chemical Analysis

Methodology Code: Data Analysis and Manipulation
A large proportion of analytical data acquired from Nuclear Magnetic Resonance (NMR), Liquid Chromatography (LC), and Mass Spectrometry (MS) instruments is used for routine analytical purposes such as structure confirmation and purity. In most cases the samples are relatively simple. For laboratories that acquire routine data and employ a range of instruments from different vendors the task of processing and analyzing the data is further complicated by the array of different vendors software they require. Here we present an innovative software platform that is designed specifically for routine analytical applications. It enables analytical data from a wide range of different vendors and analytical techniques to be captured and databased in a single repository. Since the data is live and accessible it can be searched and utilized within future analyses. It combines NMR, LC, MS, Optical Spectroscopy analysis and chemical structural information.

The presentation will demonstrate the platform working with several different datasets ranging from open access mass confirmation samples through to applications such as Met ID and automated structure verification by NMR. In the mass confirmation example chemical structures are utilized to further aid target identification through isotope and fragment pattern matching. Since the analytical data is accessed from a database the ability to mine this existing data will also be shown.

**Abstract Text**

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**Keywords:** Data Analysis, Laboratory Informatics, Magnetic Resonance, Mass Spectrometry

**Application Code:** General Interest

**Methodology Code:** Data Analysis and Manipulation
Today's in-service fluid analysis programs have grown significantly in the past few years with many laboratories analyzing over 2000 samples per day. Each sample requires a detailed interpretation to provide the full diagnostic potential. This interpretation includes past data about the equipment the fluid is used, plus analytical data from a variety of techniques to characterize the properties of the fluid.

Historically, combining and generating information from disparate data sets could be time consuming and daunting from an interpretation perspective. Often users would look at the overall data searching for related information. With today's sample loads and volume of data, revealing information using these traditional approaches are unmanageable.

This presentation will discuss the latest tools for in-service fluid analysis data interpretation. We will discuss the fusion of data sets, in particular FT-IR and ICP and novel ways of looking at, cross-correlating, statistically processing and visualizing the relationships between the data to reveal more information.

Keywords: Chemometrics, Data Analysis, Petrochemical
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Data Analysis and Manipulation
The Royal Society of Chemistry provides open access to data associated with tens of millions of chemical compounds. The richness and complexity of the data has continued to expand dramatically and the original vision for providing an integrated hub for structure-centric data has been delivered across the world to hundreds of thousands of users. With an intention of expanding the reach to cover more diverse aspects of chemistry-related data including compounds, reactions and analytical data, to name just a few data-types, we are in the process delivering a Chemistry Data Repository. The data repository will manage the challenges of associated metadata, the various levels of required security (private, shared and public) and exposing the data as appropriate using semantic web technologies. Ultimately this platform will become the host for all chemicals, reactions and analytical data contained within RSC publications and specifically supplementary information. This presentation will report on the challenges of managing “Big Data” for chemists around the world and providing access to tools for structure dereplication, spectral database searching and the crowdsourcing of the worlds’ largest spectral database.

Keywords: Data Analysis, Informatics, Laboratory Informatics
Application Code: General Interest
Methodology Code: Laboratory Informatics
The Eureka Research Workbench (Eureka) is an open source electronic laboratory notebook and research management system targeted at academics. Eureka provides a basic infrastructure for capturing research activities with a plugin architecture to add additional functionality as needed by the users.

In this presentation we will discuss the development of a synchronous data ingest plugin that utilizes the cloud based Dropbox service. Once installed and configured data recorded on connected instruments are automatically saved to Dropbox and using the Dropbox API, Eureka is notified and automated ingestion occurs. Pros and cons of this approach will be discussed, as well as additional automated actions and can be added to this process.

Keywords: Informatics, Laboratory Informatics
Application Code: Laboratory Management
Methodology Code: Laboratory Informatics
The field of Laboratory Informatics is rapidly evolving with expanding functionality and fundamentally new tools which are charging how laboratories operate and deliver value to customers across all industry and laboratory sectors including health care, food, consumer, forensics, automotive, chemical, energy, manufacturing, mining, government / regulation, defense, nuclear and academic.

Supporting Laboratory Informatics standards are also evolving both within the laboratory and between external systems. Laboratory Informatics provides a vital link in the capture, processing, trending and reporting of information.

The focus of this discussion is on the newly approved ASTM E1578 Laboratory Informatics Standard Guide.

The scope of ASTM E1578 Laboratory Informatics Standard Guide was broadened to include the primary tools in today’s laboratory informatics area (examples include: Laboratory Information Management Systems (LIMS), Chromatography Data Systems (CDS), Electronic Laboratory Notebooks (ELN) and Scientific Data Management Systems (SDMS). Additional terms related to laboratory informatics are now defined and new sections, including one on lean concepts and knowledge management.

A wide segment of laboratory informatics users, vendors and interested stakeholders participated in the recent revision of the standard. The primary audiences of the standard include: end users of Laboratory Informatics tools, implementers of Laboratory Informatics tools, information technology personnel, Laboratory Informatics tools vendors, instrument vendors, individuals who approve Laboratory Informatics funding, Laboratory Informatics applications support specialists and software test/validation specialists.

Keywords: Lab Management, Laboratory Informatics, LIMS, Scientific Data Management
Application Code: Laboratory Management
Methodology Code: Laboratory Informatics
Data Manipulation and Laboratory Informatics

Bringing the Quality Laboratory into the Enterprise

QA/QC laboratories in organizations that use SAP® are facing the need to implement tighter integration between the data that they are producing and the enterprise system. In some cases the organization is looking to further leverage their investment in SAP by using SAP QM as a replacement for LIMS or it may be part of a program to consolidate technologies across the organization in order to reduce IT costs. This presentation will explore examples of laboratories who have integrated with SAP providing a positive impact on operational excellence in the laboratory as well as the enterprise.

Keywords: Biopharmaceutical, Data Analysis, Data Mining, Informatics
Application Code: Quality/QA/QC
Methodology Code: Data Analysis and Manipulation
Hydrogen sulfide, along with carbon monoxide and nitric oxide, is a gaseous signaling molecule known as a gasotransmitter. All of these molecules help regulate ion channels within the body, however there is a particular desire to selectively detect H$_2$S in vivo due to its physiological significance in the central nervous and cardiovascular systems. Independently detecting H$_2$S in the presence of NO and CO will allow for the full understanding of both its individual physiological roles as well as its interactions with NO and CO.

Unfortunately there is currently no method that allows for selective in vivo detection of H$_2$S. One possible method is to design an amperometric gas sensor which incorporates an electrocatalytic material that selectively oxidizes H$_2$S in the presence of NO and CO. Our group has been actively investigating cyanide coordinated to electropolymerized ferrirrotoporphyrin (FePP) as such a material, which may be able to be incorporated into a future sensor design. However, the electropolymerization of FePP on Pt microelectrodes did not previously yield a layer with adequate selectivity for practical applications. This presentation will report our efforts in understanding and improving the FePP electropolymerization process, specifically with regards to optimizing the electrochemical reduction of graphene oxide in order to serve as a potential FePP support material. This work is sponsored through an award from the National Science Foundation.
Tear glucose measurements have been suggested as a potential alternative to blood glucose monitoring for diabetic patients. While previous work has reported that there is a correlation between blood and tear glucose levels in humans,1-3 this link has not been thoroughly established and additional clinical studies are needed. We previously reported that Roche’s electrocemical Accu-Chek glucose test strips exhibit far superior analytical performance over other commercial brands with the low detection limit and high selectivity required for quantitating tear glucose.4 Herein, we evaluate the origin of the high sensitivity and selectivity of the Roche test strips and show that the use of pyrroloquinoline quinone (PQQ)-dependent glucose dehydrogenase (GDH) as an active enzyme reagent is responsible for the low limit of quantification (ca. 9 \( \mu \text{M} \)). Further, it will be shown that the combination of a gold working electrode and a nitrosoaniline derivative as an electron transfer mediator is responsible for the high selectivity over ascorbic acid, uric acid and acetaminophen observed when using these test strips. The excellent analytical performance of these strips enables them to be applied for measurement of glucose levels in basal or reflux tears of both normal and diabetic human subjects to further understand the clinical utility of the glucometer test-strip method.

Literature cited:

Keywords: Bioanalytical, Biomedical, Biosensors, Electrochemistry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
In this talk we report on a paper sensor for antibody detection in whole blood. The sensor uses the principle of target-induced dynamic switching to activate electrochemical detection. We have previously demonstrated the effectiveness of this type of paper sensor (Scheme 1) for detection of DNA and thrombin down to 30 nM and 16 nM, respectively, with device-to-device reproducibility below ±10% and a shelf life of at least 4 weeks [1].

As shown in Scheme 2, the new antibody esensor comprises electrode-bound oligonucleotide duplexes that have a pendant redox reporter (in this case methylene blue) and a relevant recognition element (in this case digoxigenin or dig for short) on the distal ends. In the absence of anti-dig antibody binding, the flexibility of the scaffold yields efficient electron transfer. When the antibody binds, there is a decrease in collision dynamics and therefore faradaic current, which is easily detected using alternating current voltammetry (ACV).

The data in Figure 1 shows that the initial peak current of 0.33 A decreases by 30% after addition of 30 nM anti-dig antibody. By integrating a blood separation membrane [2] with our current esensor platform we can filter out interferences from whole blood and detect a specific antibody within a single finger prick (~15 μL).

To summarize, we have reported a paper-based esensor for detection of proteins, DNA, and antibodies that is based on the principle of target-induced conformational or dynamic switching. This is a remarkably simple and robust approach to biosensing, and consequently it is especially well-suited to the equally simple and robust characteristics of the type of paper-based device described here. Moreover, the esensor is readily adaptable to other types of redox beacons, and hence it seems likely that it could be used as a commercial point-of-care sensor for a variety of targets.

Keywords: Biological Samples, Biosensors, Electrochemistry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Electrochemical Sensors for Bio-Analysis

Electrochemical Microfluidic Immunoarrays for Assessment of Non-Aggressive vs. Aggressive Forms of Prostate Cancer Using Panels of Protein Biomarkers

Prostate cancer is a leading cause of death among men in the United States and throughout the world. Current prostate cancer detection and staging strategies often fall short in terms of specificity, and ability to clearly identify aggressive forms of the cancer. This often results in unnecessary treatments that can adversely affect the patient’s quality of life and increases healthcare costs. Assaying panels of biomarkers that are specifically involved in the pathobiology of prostate cancer holds enormous potential for not only diagnosis but also may give information regarding both the grade as well as the spread of the disease. Described here is a semi-automated microfluidic system for the on-line capture and detection of a panel of four biomarker proteins (CD-14, ERG, IGFBP-3 and GOLM-1) that have been reported to be involved in pathobiological model of prostate cancer. These protein analytes are captured from serum samples in an online microfluidic chamber by heavily enzyme- and antibody-labeled magnetic beads. Captured analytes are then magnetically separated, washed and introduced into a detection chamber housing an array of 8 nanostructured, antibody-labeled electrodes. Using this strategy, extremely low detection limits (fg mL^{-1}) have been achieved for each of these protein biomarkers. In order to accomplish multiplexed detection of these biomarkers in clinically relevant ranges, we tailored the dynamic range by controlling assay times. The measurement of these small panels of selective biomarkers holds remarkable promise for future cancer diagnostics and personalized cancer therapies.

Abstract Text

Keywords: Bioanalytical, Electrochemistry, Immunoassay, Sensors

Application Code: Bioanalytical

Methodology Code: Electrochemistry
The biofilm architecture of *Pseudomonas aeruginosa* has been widely studied because it represents a major problem in oil, gas, and shipping industries, as well as in sewer lines, polluted coastal waters, and medical devices. Recent evidence from Dr. Sauer’s laboratory proved that during biofilm formation, pyruvate was released by resident bacteria. Additionally, the self-fermented pyruvate support further biofilm growth. Conversely, the depletion of pyruvate significantly impaired biofilm growth. Similar results were found in *Escherichia coli* (Gram-negative bacterium) and *Staphylococcus aureus* (Gram-positive bacterium). This evidence suggested that self-produced pyruvate, which could be detected in supernatants, is a fundamental indicator of biofilm colonization and development. In this work, novel biosensor for pyruvate was constructed using Pyruvate dehydrogenase (PDH) entrapped within conducting polypyrrole membrane, which was electrochemically-deposited onto microelectrode arrays with polyethylene terephthalate (PET) platform. PET substrate allows this sensor to be easily applied onto wound detection. The immobilized PDH catalyzed the conversion of pyruvate into acetyl CoA and NADH. This reaction was monitored electrochemically via a mediator. Preliminary results showed the linear range was between 1 mM to 2.5 mM with $R^2=0.9932$.

**Abstract Text**

**Keywords:** Biosensors, Electrochemistry

**Application Code:** Bioanalytical

**Methodology Code:** Sensors
All cells in the body continually exchange various ions with the external fluid (e.g., cellular fluid, blood, sweat and blood) surrounding them. This external fluid exchanges ions such as calcium, sodium and potassium with the blood being pumped throughout the body. Hence, the chemical composition of the external fluid is extremely important for the cell.

Ion concentrations in the external fluid are commonly measured by use of benchtop analyzer system for determining consumption and production of metabolites. However, this method are not appropriate for on-site measurement and monitoring because this system is too large to carry out, so it is difficult to easily and quickly measurement of changing chemical composition of the external fluid.

In this presentation, we propose new measurements by use of flat ion selective electrodes. By use of these instruments, we can easily and quickly measure ion concentrations of the external fluid.

Keywords: Bioanalytical, Electrochemistry, Electrodes
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Solution and surface conjugation, or coupling, between substances with carboxylic and amino-functional groups, is very important in areas of e.g., bioconjugate, biosensing, and biosynthesis. We report herein the systematic electrochemical studies of the surface coupling under various experimental conditions. A monolayer of 1,7-diaminoheptane (diamine) or 4-aminobenzoic acid (4-ABA) was first electrochemically oxidized and deposited on the surface of a glassy carbon electrode, which subsequently reacted with an electroactive compound, ferrocene acetic acid or aminoferrocene, respectively, to form the surface-confined peptide bond under various buffers in the presence of the crosslinking agent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC). The coupling efficiency, which was proportional to the amount of ferrocene moiety (Fc) covalently attached to the diamine or 4-ABA, was finally evaluated with cyclic voltammetry (CV), where the CV current of Fc directly corresponded to the number of peptide bond formed on the electrode surface. Effects of pH value of the reaction media, buffer composition, EDAC concentration, reaction time, and concentration of Fc on peptide bond formation will be discussed.

Financial support from the NSF CAREER Award (CHE-0955878) and the Eagle SPUR grant from the University of Southern Mississippi (JG) is gratefully acknowledged.
The integration of biological building-blocks with synthetic nanomaterials may permit unprecedented ability to detect, disinfect and completely remove pathogens in water. We described the synthesis of biodegradable, interpenetrating polymeric networks of poly (amic) acid (PAA), glutaraldehyde-derivatized PAA (PAA-GA) and chitosan-modified poly (amic) acid (PAA-CS) using phase-inversion procedures. The characterization data from NMR, FT-IR, SEM and cyclic voltammetry confirmed the successful formation of electroactive, bifunctional, glutaraldehyde-linked PAA membranes. Toxicological, electrochemical and mechanical characterization data showed the successful formation of non-toxic, biodegradable, porous, free-standing and mechanically nanostructured membranes. PAA-GA showed the highest modulus of 568.1 Mpa followed by PAA-CS-GA (495.0 Mpa). The optimized membranes were tested against three of the most common drinking water contaminants, namely Escherichia coli, Citrobacter freundii and Staphylococcus epidermidis with 100% removal achieved using dead end filtration and tangential flow filtration.
Phenols are classified as priority pollutants and endocrine disrupters. Initial study of water systems in Cape Town indicated their presence in potable waters and in some fresh- and wastewaters. Their presence in potable wasters indicated current problems in the city’s water treatment processes in removing these compounds because of their recalcitrant nature. Advanced oxidation processes (AOP) using O3 / H2O2 has been a possible chemical remediation route and in this work the focus was on the use of AOP enhanced by the use of iron nanorods (FeOOH nanorods). We report the catalytic properties of hydrothermally synthesised ultra-small FeOOH nanorods in the ozonation degradation of 4-Chlorophenol (4-CP). XRD, TEM, EDS, SAED, FTIR and BET were used to characterize the prepared material. Interaction between O3 – FeOOH was evident from the FTIR results and was attributed to the high surface Lewis-acid sites of the ultra-small FeOOH nanorods. The catalytic properties of the material during ozonation were found to be pronounced at lower initial pH of 3.5. Two stage first order kinetics was applied to describe the kinetic behaviour of the nanorods at low pH. We also report the catalytic oxidation of 4-chlorophenol. The first stage of catalytic ozonation was attributed to the heterogeneous surface breakdown of O3 by FeOOH, while the second stage was attributed to homogeneous catalysis initiated by reductive dissolution of FeOOH at low pH. The process was found efficient for the remediation of 4-chlorophenol in water systems.

Keywords: Environmental/Water, Liquid Chromatography/Mass Spectroscopy, Nanotechnology
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
Osorb media, an organically-modified silica adsorbent that is applied to water treatment applications, is also capable of regeneration with little loss of efficiency. One use of the media is for organic removal from oil and gas produced water streams as a final polishing step before discharge or reinjection into formations. The media is capable of reducing oil in water levels to below environmental regulations. If regenerated, the media is more cost effective than existing technology. Various regeneration methods are required depending on the type of organics the media has captured. This research focuses on the testing of Osorb media that has captured organics from produced water streams and then been regenerated, for its sustained efficiency at oil removal and its capacity for dissolved organics such as benzene, toluene, ethyl benzene, and xylenes (BTEX). Efficiency was evaluated by determining the percentage of oil removed by the regenerated media from an aqueous solution. Oil concentration was established using solvent extraction followed by infrared spectroscopy analysis. Capacity was assessed by flowing a saturated BTEX solution through fixed bed of regenerated media over a period of time. The BTEX concentration was calculated using solvent extraction followed by analysis on the gas chromatographer mass spectrometer. The capacity and efficiency of regenerated Osorb media was compared to new media to determine if regeneration was successful. We are presenting two methods that were able to return up to 90-95% of the efficiency and 50-95% of the capacity after media regeneration.
High concentration of ammonia can complicate the chlorine-disinfection process. When adding sufficient chlorine to achieve breakpoint chlorination for ammonia removing, high levels of DPBs (THMs and HAAs) will be produced. In addition, if N-nitrosamine precursors present and react with monochloramine (MCA) and/or free chlorine, highly toxic N-nitrosamines, mainly NDMA, will be formed. The objective of this study is simultaneous removal of N-nitrosamine precursors and ammonia by using zeolite. Six-gang stirrers were utilized to simulate the water treatment process of water treatment facilities. Zeolite was added at the rapid mixing step and then proceeded through flocculation and sedimentation. The effects of lime softening, sweep flocculation, pH, zeolite dosage, and contact time on the removal of ammonia and N-nitrosamine precursors by zeolite were all evaluated. In addition, the removal efficiencies of ammonia and N-nitrosamine precursors by zeolite with and without the presence of monochloramine and/or free chlorine were also investigated. A UFLC-MS/MS method recently developed in our laboratory was used for quantitative measurements of N-nitrosamine precursors and HACH kit was used for free ammonia measurement. Results showed that the Zeolite removed N-nitrosamine precursors and ammonia effectively, and also reduced the formation of THMs and Nitrosamines. The detailed experimental conditions and the results will be presented at the conference.

This study was supported by Missouri Department of Natural Resource.
Objective: To provide an overview of instrumental methods to analyze the off-flavors in aquaculture products.

Significance: Musty, muddy, off-flavors have been reported in farm-raised sturgeon, large mouth bass, tilapia, trout, salmon, barramundi, and catfish. The off-flavor metabolites 2-methylisoborneol and geosmin are produced by cyanobacteria and actinomycetes. Off-flavor occurs in open ponds, recirculation systems, and even in open bays and rivers. Some industries employ professional flavor checkers to limit the amount of off-flavor product from reaching the markets. Instrumental analysis can give a more accurate and precise result but is slower and more costly.

Experimental: Analysis of 2-MIB and geosmin in water is accomplished using GC/MS with SPME, purge and trap, or solvent extraction. An electronic nose has also been evaluated. Analysis in tissue is more problematic as the off-flavors tend to reside in the fat and are more difficult to extract. Tissue is generally homogenized with sample size ranging from 5 to 50 g. Solvent extraction and Microwave desorption/SPME of the tissue with subsequent analysis by GC/MS are the most prevalent methods for analyzing fish.

Results: Several factors influence the levels measured by these methods, including sample size, cooking time, temperature, fat content and possible compounds that affect the matrix. This talk will explore the strengths and weaknesses of the different analytical methods that are used to measure off-flavor compounds in aquaculture products.

Conclusions: This research provides qualitative information on the compounds found in the headspace of cooked fish and provides details for the quantification of 2-MIB and geosmin. These compounds can be detected by humans at concentrations as low parts per trillion ranges in water and at the sub parts per billion ranges in fish.

This research was funded by the USDA-ARS CRIS Number 06435-44000-077-00D

Keywords: Gas Chromatography/Mass Spectrometry, SPME
Application Code: Other
Methodology Code: Gas Chromatography/Mass Spectrometry
In the study of an off-color problem with catfish fillets, the carotenoids causing the yellow-color problem needed to be isolated, identified, and quantified. The isolation of the carotenoids from the triacylglycerols, comprising the bulk of the oil extracted from the fillet, was explored by the use of solid phase extraction (SPE). Both silica and amine phase sorbents were tested. For the separation, identification, and quantification of the isolated carotenoids, high performance liquid chromatography (HPLC)-photodiode array (PDA)-mass spectrometry (MS) was used. For the chromatography, a comparison of C18, phenyl-hexyl, silica, and C30 column phases were investigated. For MS detection, electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photo ionization (APPI) sources were tested in both positive and negative ionization mode.

The four xanthophylls, lutein, zeaxanthin, diatoxanthin, and alloxanthin, in addition to two tentatively identified cis-isomers, 9'-cis lutein and 9-cis zeaxanthin were best separated and identified by LC-MS using silica chromatography and APPI positive and negative mode or APCI negative mode ionization. The C30 column also separated the four xanthophylls to a lesser degree, but no separation occurred with C18 or phenyl-hexyl columns. Protonated molecular ions [MH]+ were observed from the C30 column with ESI, but only non-protonated molecular ions [M]+ occurred with silica chromatography. Also noted was the unusual identical elution order from the normal (silica) and reversed-phase (C30) columns. The results show silica-APPI-MS as a possible improvement to the normal C30-ESI-MS method used for carotenoid identification.
Rice bran contains several bioactive components that have been linked to the promotion of human health. Brown rice bran contains lipophilic components that include the tocotrienols and oryzanol. Pigmented or colored rice bran contains different phenolic compounds including several anthocyanins in purple (black) rice. Some of these bioactive compounds individually have been shown to promote glucose uptake and aid in glucose homeostasis in animal studies. However, only recently has rice bran been examined for its potential to aid in glucose management. The objectives of this study were to: evaluate glucose uptake using 3T3-L1 cells and determine the anthocyanin composition of purple rice bran extracts.

Several commercially available rice varieties were chosen for this study. The brown rice bran variety Cocodrie was used in the glucose uptake assay for comparison. The purple rice used in this study were Blanca Isabel (Rush Rice Products), Forbidden (Lotus Foods) and Black Japonica (Lundberg Farms). Rice bran will be milled directly before use. Bran samples for glucose uptake will be extracted with 70% ethanol, dried, and reconstituted in DMSO (100 mg/mL). Rice bran extracts were applied to mature 3T3-L1 adipocytes 18h before glucose uptake determination using [3H]-2-deoxy-D-glucose. For quantitation of anthocyanidins, bran samples will be hydrolyzed with hydrochloric acid and quantitated by UPLC using available standards. LC-MS using an APCI source will be used to identify anthocyanin composition.
The incidence of peanut allergy continues to rise in the US and Europe. Whereas exposure to the major allergens Ara h 1, 2, 3, and 6 can cause fatal anaphylaxis, exposure to the minor allergens usually does not. Ara h 8 is a minor allergen. Importantly, it is the minor food allergens that are thought to be responsible for Oral Allergy Syndrome (OAS) in which sensitization to airborne allergens causes a Type 2 allergic reaction to ingested foods. Furthermore, it is believed that similar protein structure, rather than a similar linear sequence is the cause of OAS. Bet v 1 from birch pollen is a common sensitizing agent and OAS results when patients consume certain fruits, vegetables, tree nuts and peanuts. Here, we report the 3-dimensional structure of Ara h 8, a Bet v 1 homolog. The overall fold is very similar to that of Bet v 1, Api g 1 (celery), Gly m 4 (soy) and Pru av 1 (cherry). Ara h 8 binds the isoflavones quercetin and apigenin, as well as resveratrol avidly. Using micro-chip technology we have mapped the important linear epitopes for IgE binding.
Foods produced in large scale frying operations are heavily impacted by the condition of the frying oil. Color, texture and flavor of fried foods are greatly influenced by the condition of the frying oil, and the oil itself represents a significant portion of the production cost of the fried food. Decisions on whether to replace the oil, add new oil or continue using the oil currently in the frying system impact sensory characteristics of the final product, and can have a significant impact on profitability. Laboratory methods employed to assess quality factors in frying oils include determination of Free Fatty Acids (FFA), p-Anisidine Value, Total Polar Compounds and Polymerized Triglycerides. Each of these tests are performed as individual tests and employ reagents that require proper disposal, training of laboratory personnel, and labor to perform each of the tests.

In this paper we will present the use of FT-NIR to determine of quality factors in frying oils. FT-NIR calibrations for FFA, IV, p-Anisidine Value, Total Polar Compounds and Polymerized Triglycerides are shown provide accuracy comparable to the standard error of the laboratory methods. Additional benefits of the FT-NIR method include the ability to perform all measurements using a single sample in less than 2 minutes, having a measurement precision significantly better than the repeatability of the individual laboratory methods, and require no chemicals or disposal fees. Accuracy, precision and speed of the FT-NIR method make it an ideal choice to monitor the condition of frying oils and enable plant personnel to make informed decisions to maintain cost controls and sensory qualities for the products they produce.

Keywords: Food Science, Near Infrared
Application Code: Food Science
Methodology Code: Near Infrared
In recent decades, the vitamin supplement product market has grown to a multi-billion dollar business. As the market grows rapidly, various types of vitamin supplements have been introduced to the market. Due to the increasing diversity of product matrices as well as its complexity of formulations of active components, preservation of those active components against any chemical reactions such as oxidation and interactions among active components has become more challenging than ever.

Liquid Chromatography Quadruple Time-of-Flight Mass Spectrometry was used as a forensic tool to investigate the cause of potency loss through profiling of degradation products under various conditions such as heat, oxidation, acid, base, and interaction with other components. LC-QTOF-MS was used to separately identify and profile Cyanocobalamin (B12) and Cholecalciferol (D3) degradation products. Based on published literatures, known degradation products of the vitamins were identified by matching molecular ions and sodium adduct ions. Also, unknown degradation products with accurate mass ions plus retention times on a chromatography system were successfully classified as a fingerprint against stress conditions with a good reproducibility. Finally, the practicality of the degradation product profiles by LC-QTOF-MS was successfully demonstrated for experimental formulations containing Cyanocobalamin and Cholecalciferol through identification of the cause of potency loss.

Keywords: Liquid Chromatography/Mass Spectroscopy
Application Code: Food Science
Methodology Code: Mass Spectrometry
Quality by Design (QbD) has been well established in the pharmaceutical industry for manufacturing processes [ICH Q8(R2)]. It is defined as a systematic approach to process development that begins with predefined objectives and emphasizes product and process understanding as well as process control, based on sound science and risk management. The QbD concept for process development can also be applied to the development of analytical methods in the laboratory. A major advantage of applying the QbD concept to analytical method development is enhanced robustness of the resulting method that meets all critical performance requirements during the method development.

The QbD concept was used to determine the relationship among various critical factors (UPLC column type, pH, hydrophobicity, gradient time) against the chromatographic resolution of water soluble vitamins in dietary supplement softgels and tablets during the long-term stability studies. Four different types of UPLC columns were evaluated at three different pH conditions (pH 2.0, 4.0, and 6.0), using acetonitrile as the organic solvent. Based on information obtained from the screening runs the effects of the major factors affecting chromatographic peak resolution were determined, and the operating space was established.

Keywords: Chromatography, Food Science, HPLC, Method Development
Application Code: Food Science
Methodology Code: Liquid Chromatography
Nutritional supplements can be effectively used to improve the nutrient status of many individuals. Robust and accurate analyses of vitamins for dietary supplements are required, particularly when working with micro-vitamins (Folic acid, Biotin and Vitamin B12) in multi-vitamin matrices. Because of the inherent complex nature of multi-vitamin matrices, and the added complexity due to the presence of flavors, using conventional HPLC-UV methods with an ion-paired column, the analyses of these compounds become more challenging than ever. The objective of this study was to develop a robust LC-MS method using LC-QTOF and QDa for the quantitation of Folic acid, Biotin and Vitamin B12 in dietary supplements using isotopic internal standard spiking.

LC-QTOF method in combination with a constant level isotope spiking was developed as a reference method for the accurate determination of micro vitamins. Each injection (standard and sample) was spiked with the respective isotope standard to determine the dynamic linear range and to compensate for ion suppression and/or enhancement. The injector program was used to spike samples with isotopic internal standard. The linearity, accuracy, precision and reproducibility of the method was evaluated.

This presentation covers the results obtained from the LC-QTOF using isotopic internal standard for Folic acid, Biotin and B12 and the results show how the LC-QTOF method can be adapted for routine analysis.

Keywords: Chromatography, Liquid Chromatography/Mass Spectroscopy, Method Development, Quadrupole M
Application Code: Food Science
Methodology Code: Liquid Chromatography/Mass Spectrometry
There are many volatile compounds in wine that help define the complex sensory effects of wine. These compounds contribute to aroma character of wines such as fruity, vegetal, butterscotch, leathery etc. We analyzed several of these aroma compounds using a GC-quadrupole time of flight (q-TOF) mass spectrometer with a headspace autosampler. Target analytes were selected in the mass discriminating quadrupole of the qTOF, fragmented in the collision cell, and the spectral fingerprint generated was analyzed by the TOF end of the mass spectrometer. The MS/MS data generated for the target analytes was used to confirm the presence of these analytes. The GC-qTOF is a powerful technology to obtain high selectivity for identification of target analytes with full spectral information without losing sensitivity.

**Keywords:** Flavor/Essential Oil, Food Science, Gas Chromatography/Mass Spectrometry

**Application Code:** Food Science

**Methodology Code:** Gas Chromatography/Mass Spectrometry
The effect of different doses of Baccaurea angulata (BA) whole fruit juice (0, 0.5, 1.0, and 1.5 mL of juice per kg adult rabbits per day) given to high-cholesterol fed rabbits for 90 days was studied. The work was carried out to detect an accumulation of fatty streak, evaluate the percentage of atherosclerotic lesion accrued and intima-media thickness in the aorta of normo- and hypercholesterolemic rabbits. The result showed that the supplementation of high-cholesterol diet of hypercholesterolemic rabbits with only 0.5 mL BA/kg rabbit per day significantly (p<0.001) attenuated aortic fatty streak development. The treatment with BA fruit juice also significantly (p<0.001) reduced the atherosclerotic lesion compared to the negative control group, NgC (94.37±1.09%). The various doses of BA at low, medium and high levels (LD, MD and HD) gave percentage reduction in the atherosclerotic lesion of 53.85±1.48, 28.13±1.13 and 11.39±1.13% respectively. There was significant (p<0.001) less thickening of tunica intima to tunica media ratio in the low dose, medium dose and high dose groups (1.60±0.10, 0.25±0.05 and 0.10±0.06 respectively) compared to NgC (2.37±0.06). Higher BA doses used (1.0 and 1.5 mL/kg rabbit per day) also significantly decreased further the development of aortic fatty streaks. Therefore, Baccaurea angulata possesses antiatherosclerotic potential benefit.
Oxidation in hydrocarbons often begins with an external stress. This stress can be associated with mechanical fatigue, ultraviolet radiation, or heat. This results in the formation of a free radical which is susceptible to oxygen insertion and results in the formation of a carboxylic acid. Thermal Analysis in conjunction with Infrared Spectroscopy provides a unique vantage point to study the formation and propagation of oxidation by tracking changes in functional groups, heat flow, and weight loss. In this study, a series of edible oils will be characterized by their oxidative and thermal stability under inert and oxidizing atmospheres through Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA). Olive, coconut, canola, peanut, vegetable, sesame, grape seed, and corn oil will be the targets of this experimentation. A select few of the oils will also be subjected to pre and post oxidation testing by Fourier Transform Infrared Spectroscopy (FTIR), as well as the hyphenated technique of TGA-FTIR. Oxidative onset temperature by DSC (ASTM E 2009) will be one of the techniques utilized in the study. It will be shown that while some oils (coconut) have a significantly higher oxidative stability their overall thermal stability is considerably less than expected, especially in the presence of an oxygen-rich atmosphere. Principle Component Analysis (PCA) of the TGA-FTIR data will also be demonstrated as a tool to track decomposition within a material.

Keywords: FTIR, Materials Characterization, Thermal Analysis
Application Code: Food Science
Methodology Code: Thermal Analysis
Comprehensive two-dimensional gas chromatography (GCxGC) is recognized as the most powerful tool for the separation of complex mixtures of volatile and semi-volatile compounds. However, less than ideal chromatography is often produced when one utilizes GCxGC for the analysis of highly concentrated or active analytes. GCxGC chromatograms of such samples typically contain broad, tailing analyte bands. This results in difficulties with quantitation and poor utilization of the separation space. To investigate the source of these tailing bands, we studied the inlet and modulator as potential contributors. A simple inlet flushing device was developed to isolate the injector from the primary column after the injection, and a similar setup was used to isolate the modulator from the primary column. This device allows the user to divert carrier gas flow back through the injection port at a specified time after the injection, while allowing analytes to pass through the column for separation. Analytes retained within the injection port are prevented from entering the column, and are subsequently removed via the carrier gas split line. The study revealed that the injection port plays a critical role in the development of tailing chromatographic bands, while the modulator simply modulates the already tailing band. Using the device, GCxGC chromatography in several applications was improved. The design of the device, its performance and applications will be discussed.

**Keywords:** GC, Instrumentation, Separation Sciences

**Application Code:** General Interest

**Methodology Code:** Separation Sciences
Previous research in our group has demonstrated the power of thermodynamic-based predictions of GC retention times, in both GC and GCxGC modes. Retention time accuracies are generally better than 0.5-1 s in 30-40 min in GC mode and within a few 10s of ms for the second dimension in GCxGC mode. More recently, we demonstrated a method whereby the thermodynamic parameters for a given molecule on a particular stationary phase could be extracted from a suite of 5 temperature-programmed GC separations. This permits the acquisition of thermodynamic data to be accelerated to upwards of 500 molecules per week.

With these successes, one key factor of the separation prediction is missing: peak widths. Herein, we present improvements to our predictive model that permit the width of GC (or GCxGC) peaks to be predicted. This is a crucial step in the development of optimization software which requires a prediction of chromatographic resolution for a pair of peaks. This is even more important in developing GCxGC methods as insight into the peak widths of analytes is required, not only to evaluate the resolution between peaks, but also in order to automatically optimize the modulation ratio and modulation period for the separation.

Keywords: Capillary GC, GC, Other Hyphenated Techniques
Application Code: General Interest
Methodology Code: Gas Chromatography
The impetus for the measurement of low levels of sulfur compounds has come from the hydrocarbon process industry, where the requirement for lower sulfur measurements is steadily increasing. Common sulfur impurities in the feedstock, such as H2S (hydrogen sulfide) must be carefully monitored. To monitor, control, and regulate compound, calibration gas mixtures must be used. For H2S standard gas to be utilized successfully, it must be reliable with respect to stability (shelf life) of concentration. Significant challenges arise as the stability of the concentration of the reactive gas mixtures must remain constant, even as the cylinder pressure is diminished. Interaction on the cylinder surface, chemical reaction and adsorption, are often insignificant, with reactive gas concentrations in the range of 10ppm and above. These interactions become quite important with reactive gases at concentrations at or below 1ppm. Typically as the cylinder pressure decreases to as low as 3MPa, the H2S concentration tends to change. Since accurate and reliable calibration mixtures are important for process evaluations as well as regulatory monitoring, an experimental study was designed to assess the effect of cylinder pressure on the stability of the H2S concentration. Various low concentration H2S mixtures (1,5,10ppm) in balanced with nitrogen were filled in specially treated cylinders and each concentration was monitored as a function of pressure from 8MPa down to 1MPa. H2S was analyzed with GC equipped with a sulfur chemiluminescence detector. A Japanese accredited SO2 mixture was used to quantify H2S concentration indirectly.

This presentation will illustrate the necessity of suitably treated cylinder surfaces to achieve uniform concentrations throughout the cylinder depletion.

**Abstract Text**

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This presentation will illustrate the necessity of suitably treated cylinder surfaces to achieve uniform concentrations throughout the cylinder depletion.

**Keywords:** Specialty Gas Analysis

**Application Code:** Environmental

**Methodology Code:** Gas Chromatography
Gas chromatography-mass spectrometry (GC-MS) is probably the most widespread tandem technique in the analytical laboratory today and deployed into nearly every industry. While MS provides a fast, sensitive and qualitative detection system for GC, it suffers from many disadvantages. These include varying detector responsivities requiring constant calibration, non-linear calibrations (poor dynamic range), difficulty in measurement during injection peak, solvent elution or elution of high concentration analytes, and an inability to differentiate many isomers. It also requires high vacuum, significant maintenance, He or H2 carrier gases and significant compound separation (to prevent interference or chemical ionization).

If a detection system for GC could provide similar levels of sensitivity with greater qualitative capabilities and much greater dynamic range while removing all the aforementioned issues it is possible it could meet the need and improve the productivity of scientists and engineers in many industries currently served by GC-MS.

We will demonstrate a new spectroscopic detection system (MAX™) for GC that can measure from parts-per-trillion to % and provide full qualification and quantification of more than 1,000 compounds during a single analysis. The MAX™ technology can be setup in a laboratory in less than a day, requires no high vacuum or expensive He or flammable H2 to operate and is not damaged if too much sample or analyte were to reach the detector. This detection system will be ideal for laboratories or businesses where productivity is improved by eliminating needless calibration or where known or unknown samples can wreak havoc with large concentration swings potentially affecting results or the operation of the analyzer.

**Keywords:** Chemometrics, Environmental Analysis, GC Detectors, Spectroscopy

**Application Code:** General Interest

**Methodology Code:** Gas Chromatography
The Proper Installation of Gas Lines for Chromatographic Equipment Can Improve Your Sensitivity, Column Life, and Increase the Safety of Handling Gases in the Lab

Your lab contains several carrier gas lines, pneumatic gases, cooling gases, and flammable gases. Discussed are recent advances in installation technics that now allows fewer components to be used in the lines and does away with the need to purge the lines with each cylinder change. These new devices allow you to maintain the purity of the gas delivered to your site all the way to your columns. This will mean longer column life and fewer very expensive column change outs. They also make the handling of high-pressure gases safer and less strenuous to accomplish.

Abstract Text

Keywords: Gas Chromatography
Application Code: Laboratory Management
Methodology Code: Gas Chromatography
Recycle Gas Chromatography with Thermal Gradient Program for Sample Re-Focus

Recycle Chromatography is a method that allows samples to pass two columns alternately many times to achieve a system with millions of theoretical plates. The separation power is high enough to facilitate separation of isotopically different compounds and isomers, which is difficult in conventional GC. However, since all the compounds of the sample must be kept in one column to avoid peak loss as the columns are switched, there is a small time window, or peak capacity. This is particularly problematic for peaks with a large R value; peak width increases very quickly within the cycle, causing a dramatic drop in peak capacity for a fixed time window. To address this situation, one of the columns has been equipped with resistive heating and a negative thermal gradient program (TGP). The TGP is capable of re-focusing the sample (that is, making a wide sample band narrower), maximizing peak capacity throughout the cycle.

Keywords: Capillary GC, Gas Chromatography, GC
Application Code: General Interest
Methodology Code: Gas Chromatography
Method development in gas chromatography has traditionally been carried out through controlled experimentation directed by chromatographic theory and the intuition of the experimenter. There are many parameters to be optimized in a modern capillary GC system including the choice of carrier gas, column stationary phase, column dimension, and temperature program to name a few. Each of these parameters can have a dramatic effect on chromatographic run time and resolution, also, they are often interdependent forcing a chromatographer to make tradeoffs in method performance.

Computer assistance can be a powerful tool in streamlining the method development process saving time, expense, and lowering the number of optimization experiments needed. A computer program will discussed that can produce accurate model chromatograms under a wide range of instrumental conditions, column dimensions, and across different chemical classes of analyte. The program can be used to obtain a good starting point for developing a new method or to optimize an existing one.

In essence a user is able to analyze the effect that changing method parameters will have on a separation without running an actual GC experiment. It will be demonstrated that the model's output corresponds well with reality, giving the user confidence that the predictions are reliable.

Keywords: Capillary GC, Chromatography, Method Development, Software
Application Code: Other
Methodology Code: Gas Chromatography
Continuous measurement of hydrocarbon gas concentration is crucial to mud loggers at the drilling site. Hydrocarbon measurement information provides operators with valuable geological formation insight during vertical, intermediate, lateral, and hydraulic fracturing drilling processes. As a result of technological advancement in gas detection, sample composition can now be measured within minutes leading to nearly real-time analysis. Gas chromatography (GC) is a well-established technique used to analyze extracted gas to provide accurate, on-site gas composition information. The INFICON Micro GC Fusion is the latest innovation that utilizes temperature programmable columns and micro-machined components to analyze C1-C8 compounds in less than two minutes or C1-C5 compounds in less than 30 seconds on a 24x7 basis. This expanded compound range and speed allow for more data to be acquired compared to traditional GC equipment. The instrument’s transportable and compact architecture makes it ideal at drilling sites where space is limited.
A new generation of Core-Shell materials were introduced in 2007 that were specifically engineered for the separation of small molecules. These materials saw the average column performance increase 30-50% over fully porous particles of similar size. Subsequent releases of these materials have made them available in particle sizes ranging from 5 µm to 1.3 µm giving average column performances between 180,000 – 450,000 plates/m. Maybe even more so than their fully porous counterparts, core-shell particles 3.6 µm and below require significantly increased linear velocities to obtain maximum resolution. While a benefit of the increased linear velocities is much faster separations, there can be drawbacks as well. A simple way to reduce flow rate is to decrease column size. The flow rate will be proportional to the column cross sectional area. The drawback, however, to decreasing column i.d., is that peak volumes also decrease. This leads to more loss of efficiency due to system dead volume contributions to peak dispersion. In this presentation we will investigate the benefits of reducing core-shell column i.d. to 1.0 mm, and contrast these benefits with the available (U)HPLC systems and their dispersion effects on column performance in this dimension.
This work reports a comparison of HPLC separations of enantiomers with chiral stationary phases (CSPs) prepared by chemically bonding brush type chiral selectors on fully and superficially porous particles (SPPs). The chromatographic performance of superficially porous CSP based columns is compared with columns packed with 5 µm and 3 µm fully porous particles (FPPs). Only one earlier example of a chiral phase being produced using SPPs and it showed that the particle properties rendered the SPPs not useful for the preparation of coated polymeric chiral selectors, resulting no improvements in resolution. However, theoretical treatments indicate that SPP based CSPs may show increases in resolution. Herein, true gains in efficiency and resolution are obtained using SPP CSPs. For example, when using a cyclofructan based CSP, at a flow rate of 3.0 ml/min, the number of plates on column afforded by the SPP column was ~7x greater than the number of plates on column (same length) obtained when using the 5 µm FPP based column. It was found that the selectivity and resolution of the separations were comparable between fully porous and superficially porous based columns (under constant mobile phase conditions), even though the SPP column contained lower absolute amounts of chiral selector. When tested under constant retention conditions, the SPP based CSP greatly improved resolution compared to the FPP based columns. At high flow rates the efficiency gained by using superficially porous CSP was accentuated. The advantages of columns based on SPPs become more obvious from the viewpoint of plate numbers and resolution per analysis time.

Keywords: Chiral Separations, HPLC Columns, Method Development, Separation Sciences
Application Code: General Interest
Methodology Code: Liquid Chromatography
Hydrophilic interaction liquid chromatography (HILIC) is an increasingly popular mode of liquid chromatography. HILIC uses polar stationary phases in conjunction with reversed-phase type eluents (commonly acetonitrile and water) to separate mixtures of highly polar compounds (e.g. pharmaceuticals and metabolites) [1]. HILIC retention arises mainly from partitioning of the analytes into a water layer that forms on the surface of the stationary phase [2]. Additional interactions such as ion exchange contribute to differences in selectivity [3]. In previous work, Ibrahim et al. [4] utilized a sub-set of Dinh et al.'s HILIC classification probe data to create a simple graphical means to classify the selectivity of different HILIC phases based on the hydrophilicity and ion exchange properties of the phases. This work, however, was only performed under a single mobile phase condition.

In this work the effect of different mobile phase conditions (pH and buffer concentration) on the HILIC selectivity plot was investigated. Separations of cytosine, uracil, and benzyltrimethylammonium (BTMA) were performed on several classes of HILIC columns (e.g. bare silica, amine, amide, zwitterionic) at three different pH values (3, 5, 6.8) and two different buffer concentrations (5 and 25 mM). Preliminary studies demonstrate that increased buffer concentration masks ion exchange interactions in all HILIC columns. Moreover, an increase in pH increases the cation exchange character of the column. Further investigations of these trends with additional classes of HILIC columns are currently ongoing.

It has been demonstrated that liquid water is forced out of the nanopores of hydrophobic packing materials when the pressure on the reversed-phase liquid chromatography (RPLC) columns filled with water is released. This phenomenon is a natural consequence of the existence of capillary pressure in the pores of hydrophobic materials unwetted with water and the remaining pore space is occupied by a gas phase. The fact that this phenomenon causes rather drastic retention losses for many compounds indicates that, in aqueous systems, it is the interface between the bonded layer and water that has a key role in retention.

The incorporation of a gas phase into the pores of hydrophobic materials in contact with an aqueous solution reduces the area of the water/hydrophobic interface or the volume of the interfacial water. Since the volume of the gas phase in the pores can be changed by the applied pressure, the area of the water/hydrophobic interface can also be controlled by the pressure. On the other hand, the amount of the hydrophobic moiety remains constant independent of the volume of the gas phase. This means that we are able to make a hybrid stationary phase consisting of the interfacial water, hydrophobic moieties, and gas phase, and change the structure of the stationary phase by the applied pressure. Therefore it is expected that the tunable hybrid RPLC system enables us to manipulate the separation selectivity. The fixed nanoscale gas phases may also function as the stationary phase for volatile compounds.

In the present study, we present a new type of liquid chromatography, surface bubble modulated liquid chromatography (SBMLC), that uses the stationary phase incorporated with surface nanobubbles. We will demonstrate that SBMLC is a very useful separation method that enables a sophisticated separation by manipulating separation selectivity through the pressure applied to the column.

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**Keywords:** HPLC, HPLC Columns, Modified Silica, Volatile Organic Compounds

**Application Code:** General Interest

**Methodology Code:** Liquid Chromatography
Previously in our lab, lyotropic liquid crystalline gel phases of binary mixtures of guanosinemonophosphate (GMP) with other nucleosides and nucleotides have been studied, with particular focus on mixtures of GMP with guanosine. The gels are a tunable, stable and biocompatible, offering possible applications of these materials in diverse areas such as biomedicine, pharmaceuticals, “green” architecture, and nanotechnology. From a more fundamental standpoint, the formation and characteristics of these gels could have important implications for the RNA world hypothesis in origins of life research.

Here we describe the use of NMR to study the structures resulting from self-assembly of GMP in mixtures containing other nucleotides. Previous papers have reported peaks characteristic of the formation of hydrogen-bonded G-tetrads and alternative, continuous helical structures in the 1D 1HNMR spectra of GMP. We are investigating how the addition of a second nucleotide such as adenosine monophosphate or a nucleoside such as guanosine affects the organized GMP structure. In addition to 1D 1HNMR, we are also performing 2D experiments such as Nuclear Overhauser effect spectroscopy (NOSY) and Diffusion Ordered Spectroscopy (DOSY) in order to determine the responses of the GMP spectra to the second component in the mixtures.
We will describe several sample pre-treatment approaches by sequential injection (SI) for on-line reaction monitoring by low-field nuclear magnetic resonance spectroscopy (NMR). The picoSpin™, a low-field (45-82 MHz) benchtop NMR system, was interfaced to SI for automated on-line measurements. Three SI methods were studied: (i) solid-phase extraction with a monolithic disk column, (ii) liquid-liquid extraction based upon differential flow velocities (Lucy & Yeung, Anal. Chem. 66, 2220), and (iii) membrane separation with a parallel-plate dialyzer. The results of optimization studies for each method will be presented, focusing primarily on variation of the types of stationary phases, extraction solvents, and membranes, respectively. To demonstrate the utility of the SI-NMR system, we applied the method to on-line monitoring in which a reaction vessel was sampled periodically. Specifically, we will compare the figures of merit for each of the three SI methods as applied to a study of the reaction between alpha di-carbonyl compounds and amino acids.

Keywords: Flow Injection Analysis, Instrumentation, Magnetic Resonance, Sample Handling/Automation
Application Code: Bioanalytical
Methodology Code: Magnetic Resonance
In this study the amide proton exchange rates of oligosaccharides derived from chondroitin sulfate (CS) are determined using 1H NMR and ESI MS. The aims of this work are to examine the effects of oligosaccharide structure on exchange rates and to compare the results obtained by the complementary methods of NMR and MS. CS belongs to the family of glycosaminoglycans (GAG) known for their complexity and heterogeneity, hence pure samples suitable for amide proton exchange studies are not commercially available and must be isolated in-house. Isolation of CS oligosaccharides involves the enzymatic depolymerization of the intact polysaccharide, separation by size-exclusion chromatography (SEC) into similarly-sized fractions and preparative-scale strong anion exchange (SAX) HPLC resolution and isolation of the component CS oligosaccharides from each SEC fraction. The CS enzymatic digestion was monitored by 1H NMR to obtain desired degree of depolymerization. Individual CS oligosaccharides isolated from the SAX separation were desalted and their structures elucidated by 2D NMR experiments and MS measurements. 1H NMR has been previously used to determine NH exchange rates for several GAGs and has even revealed the presence of a stable solution-state intermolecular hydrogen bond in the synthetic heparin drug fondaparinux (Arixtra). Though mass spectrometry has been primarily used to measure NH exchange rates in proteins, it should be possible to extend these studies to study the amide protons of CS and other GAGs. The results of both methods will be compared and contrasted to highlight the relative advantages of each technique in terms of the sample quantity required, experiment time, range of exchange rates that can be addressed and the robustness of the measurements.

Keywords: Chromatography, Isolation/Purification, Magnetic Resonance, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Magnetic Resonance
The chain architecture can play an important role in determining a copolymer’s physical and chemical properties. The characterization and quantification of chain microstructures (such as, monomer composition, sequence distribution, and end groups) is essential for understanding and investigating the relationship between the structures and properties of copolymers.

NMR is a powerful technology for both the qualitative and quantitative characterization of these copolymer materials. 1D $^1$H and $^{13}$C NMR spectra acquired with long relaxation delay times ($d_1 >= 5~7T_1$) can be used for the quantitative analysis of comonomer compositions and sequence distributions. $^{31}$P NMR and phosphorus derivatization are useful for quantitative determination of the functional groups such as hydroxyl and carboxylic acid end groups. 2D NMR (homonuclear and heteronuclear correlation spectroscopy) is used for the resonance assignments and structure elucidation.

$^{13}$C NMR spectroscopy is a very sensitive method useful in determining the chain microstructure of bisphenol-A based copolymers. It can, for instance, distinguish sequence compositions and their distributions up to many bonds away for bisphenol-A based co-polycarbonates. This method relies on the differences in the $^{13}$C chemical shifts of the interested carbons caused by the different sequences on their nearest neighbors in the polymer chain. This presentation will illustrate the application of this method to two commercial copolycarbonates: Bisphenol TMC (Bisphenol 3,3,5-trimethylcyclohexanone) / BPA (Bisphenol A), and PPPBP (3,3-bis(4-hydroxyphenyl)-2-phenylphthalimide)/BPA. The different diads and triads sequences can be well identified and distinguished by the resonances of carbonate carbonyls and the two quaternary carbons of the BPA.

Keywords: Analysis, Magnetic Resonance, NMR, Polymers & Plastics
Application Code: Polymers and Plastics
Methodology Code: Magnetic Resonance
# Magnetic Resonance Illicit Drug Analysis Using Benchtop NMR

Customs and postal seizures of illicit drugs expose a wide variety of illegal and “legal or not” synthetic designer drugs, structural analogues of controlled substances. An increase in the variety of designer drugs from several classes, most notably phenethylamines and cathinones, flooding borders is partly due to ambiguities in legislation surrounding the legality of structural analogues. The result is a dizzying array of structurally similar drugs that challenges presumptive testing methodologies with low discriminating power. There is a need for time-sensitive, rapid screening methods for initial drug identification with increased structure selectivity and high discriminating power to help identify designer drugs. Proton NMR spectroscopy offers a solution to the challenge by providing structure selectivity and high discriminating power in a compact benchtop instrument. Successful rapid screening identification of synthetic drugs using benchtop NMR involves the development of a spectral library of reference compounds. Characteristic NMR signatures related to the parent structure are used to discriminate both the class of compound and derivatives within the class. A standardized approach to sample preparation and analysis must consider variability of structures and composition of case samples in order to minimize the complexity of resulting spectra. Using a benchtop NMR spectrometer, an SOP was develop to acquire spectra from a series of derivatives of amphetamine and methamphetamine, and the resulting spectral library is presented. Chemical signatures are used to discriminate between the different compound classes and to identify individual compounds.

**Keywords:** Drugs, Forensics, Identification, NMR  
**Application Code:** Homeland Security/Forensics  
**Methodology Code:** Magnetic Resonance
This presentation will detail an innovative transfer system that almost entirely eliminates band spreading that generally occurs as sample materials in microfluidic/lab-on-a-chip systems pass between component devices.

Most microfluidic systems are constructed in a planar format. In this two-dimensional format, larger volume component, such as sample reservoirs, reaction chambers, and sensors, generally require the use of wide, flat geometries. To minimize turbulence during the transition of fluid in and out of these components, triangularly shaped regions are often incorporated at the ends of the component. Inlet and outlet connections are made at the apices of the triangles. It is these transition zones that introduce the band spreading. As fluid medium and entrained samples enter the component from the inlet, the fluid fans out producing a crescent-shaped fluid front across the component that persists as the fluid travels the length of the device. At the other end of the component, the fluid then funnels from across the width of the component to the outlet. As a consequence, sample particles follow flow paths of varying length. A particle on a direct line between the inlet and outlet along the center of the component travels a significantly shorter distance than one that first diverges from the inlet to the component’s width before converging at the outlet end. Path length variations of 20% are not uncommon.

This presentation will describe the progression of steps taken to address this path inequity problem and its ultimate solution. Although the presentation will concentrate on microfluidic systems, the work actually has applications almost anywhere incongruent connections are made to ribbon-shaped channels. The developed system is particularly applicable to high-throughput systems where material or sample bands must be closely spaced without significant dispersion during transport through the fluid handling system.

Keywords: Bioanalytical, High Throughput Chemical Analysis, Lab-on-a-Chip/Microfluidics, Separation Sciences
Application Code: High-Throughput Chemical Analysis
Methodology Code: Microfluidics/Lab-on-a-Chip
Graphene nanodots are considered nascent quantum dots (QDs) as they combine several favorable attributes of traditional QDs without the disadvantages of toxicity and tedious and costly synthesis. Nitrogen doped graphene quantum dots (N-GQDs) in the 4-6 nm size range, synthesized via a hydrothermal reaction between the selected carbon and nitrogen sources, have demonstrated high and stable photoluminescence (quantum yield as high as 94%), excellent water solubility, resistance to photo-bleaching, and ease of bioconjugation. These properties make N-GQDs sensitive and cost-effective substitutes for gold nanoparticles, widely-used labels in lateral flow immunoassays (LFIA). In this work, a novel lateral flow immunoassay platform was developed by applying N-GQDs for the first time as LFIA labels to target the liver cancer biomarker-α-fetoprotein (AFP). Detection limits and reproducibility for these LFIA tests were compared to those for gold nanoparticle based AFP LFIA tests. The introduction of nascent material N-GQDs into the LFIA world could open a new era for LFIA tests. N-GQDs have great potential of offering higher sensitivity and lower cost, and to be applied to medical diagnostics, forensics, therapeutic monitoring, environmental analysis, food safety monitoring and biodefense.

Keywords: Bioanalytical, Fluorescence, Immunoassay, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Microfluidics and CE

A Microfluidic Device to Measure Entrainment of Insulin Secretion from Islets of Langerhans

In vivo, insulin oscillations are found with a period of ~5 min. This oscillatory insulin signature is due to synchronization of millions of islets of Langerhans. Synchronization may occur through entrainment from in vivo glucose oscillations. Although intracellular [Ca2+] oscillations, which have been used as mimics of insulin oscillations, have been entrained to oscillatory glucose levels, there have been few demonstrations of direct entrainment of insulin secretion. To achieve this goal, a microfluidic system was developed that was capable of measuring temporally resolved insulin secretion from islets and was coupled with a gravity driven perfusion system to test if glucose waveforms can entrain insulin oscillations.

Gravity-driven flow through flow splitting channels was used to generate and deliver complex glucose waveforms to the islet chamber. This system was able to deliver sinusoidal waveforms with a period as low as 1.15 min with minimal peak broadening. Islet chamber dimensions and perfusion flow rate were adjusted for efficient sampling from single or groups of islets. Insulin secreted from islets was electrophoretically sampled and measured every 10 s with an online competitive immunoassay. This assay showed good reproducibility of the B/F with RSDs <4% and a detection limit of 2 nM.

Results showed insulin secretion from single islets was oscillatory with ~5-min periods. Both single and groups of islets were entrained to glucose sine waveforms. In addition to examining entrainment, this system will be useful for further studies of insulin secretion mechanisms and drug testing.

This work was supported by NIH grant DK080714.

Keywords: Bioanalytical, Electrophoresis, Lab-on-a-Chip/Microfluidics, Monitoring
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Separation of DNA has been central to advances in our understanding of human biology and health. Separation based on DNA fragment length alone is not enough. DNA of same length, however, often needs further analysis to determine the presence of different sequences. This can be achieved by DNA sequencing, which is becoming cheaper and faster. In some situations, however, sequencing is not practical, such as point-of-care, remote clinical settings, or rapid monitoring of changes in a pathogenic organism in response to an environmental stressor. Separation of DNA based on sequence provides a possible option. In this study, a microfluidic chip for simple, rapid, and low-cost DNA separation in two dimensions, length and sequence, was investigated. A cross channel was design and microfluidic chips were fabricated by wet etching of one piece of soda lime glass and bonded with a cover glass. Both loading and separation of samples were achieved by applying reverse voltage using a voltage supplier. The detection of signals was with the help of an inverted fluorescence video microscope. Separation of DNA in the first dimension of length was achieved using POP-4 as the matrix. For the second dimension based on sequence, separation was obtained by investigating the effects of different matrices on separation.

The research was supported by the National Institutes of Health.

Keywords: Bioanalytical, Biological Samples, Capillary Electrophoresis, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
The combination of surface-enhanced Raman scattering (SERS) and microfluidic analytics become a vivid subject due to its advantages of small sample volume and multi-function integration. In this work, we will present several microfluidic SERS chips developed, involving decorating the microfluidic channels with Ag nanoparticles contained polymer fibers by electrospinning and an ordered Ag nanodot array by depositing Ag on an AAO template and etc. In addition, we will introduce the development on the microfluidic Raman spectrometer with an inverted configuration and imaging function, which can cope with the current difficulty of focusing laser spots onto the SERS-active surface. Also, the qualitative and quantitative on-chip SERS sensing of organic pollutants and pesticides will be presented.

This work was supported by National Instrumentation Program (NIP) of the Ministry of Science and Technology of China No. 2011YQ03012408, the National Natural Science Foundation of China NSFC Grant Nos. 21373096, 21073073 and 91027010 and Innovation Program of the State Key Laboratory of Supramolecular Structure and Materials.

References

Keywords: Lab-on-a-Chip/Microfluidics, Surface Enhanced Raman
Application Code: High-Throughput Chemical Analysis
Methodology Code: Microfluidics/Lab-on-a-Chip
Microfluidic and CE

Background-Free, Multiple-Reflection Microfluidic Infrared Spectroscopy for Low-Volume, High-Sensitivity Infrared Analysis

Microfluidic technologies have shifted molecular characterization toward nanoliter volumes. Microfluidic analogues have been developed for many existing chemical techniques, including chromatography, electrophoresis, electrochemistry, and biochemical reactions. To boost the usefulness of these techniques, molecular characterization in microfluidic devices is key. Infrared (IR) spectroscopy is a clear choice, since it is highly sensitive to analyte structure and local environment. While IR spectroscopy has been successfully used in combination with these devices, to fully realize these applications, the sensing portion of the device needs to be more chemically compatible, background free, and offer higher sensitivity.

We have developed a microfluidic chip made from a broadband IR transparent crystalline material that can address all of these challenges. We demonstrate the utility of these chips by monitoring chemical reactions and protein conformation changes in IR transmission and multi-reflection attenuated total reflection (ATR) measurements. Multi-channel, multi-reflection ATR techniques will also be presented.

Keywords: Bioanalytical, Lab-on-a-Chip/Microfluidics, Microspectroscopy, Vibrational Spectroscopy

Application Code: Bioanalytical

Methodology Code: Microfluidics/Lab-on-a-Chip
Herbicides and pesticides have adverse affects on human and environmental health. Common use of these products necessitates monitoring water systems for hazardous levels due to run-off. Aptamers are an exceptional class of molecular recognition elements that selectively bind and release the target molecule. Immobilized aptamers are combined with capillary electrophoresis to create biosensors to detect herbicides and pesticides in environmental samples. Crosslinking chemistry is utilized to attach aptamers to magnetic silica particles in order to enhance detection limits through analyte concentration. With this capillary electrophoresis method, herbicides and pesticides are separated and quantified within 10 minutes. The aptamer-based biosensors are applied to detect and quantify atazine in river water [1]. In the future, the method will be translated into a portable microfluidic system for in-field analyses.


Keywords: Biosensors, Capillary Electrophoresis, Environmental/Water, Immobilization
Application Code: Environmental
Methodology Code: Capillary Electrophoresis
Autophagy is a cellular process that breaks down damaged components for recycling. Dysfunctional autophagy is implicated in the process of aging as well as various diseases including Alzheimer’s and Parkinson’s disease. Mitochondria are degraded in an organelle-specific process termed mitophagy. The individual contribution of mitophagy to global autophagy cannot be quantified by traditional analysis methods. Traditional methods used to monitor mitophagy including western blotting and fluorescence microscopy are limited by the fact that they measure organelle markers at the bulk level. Capillary electrophoresis with laser induced fluorescence detection (CE-LIF) can detect molecules at the zeptomolar level, thus detection of individual organelles with fluorescent labels is possible. This technique can monitor the number of organelles present in a sample and provide insight into their heterogeneity based on their distribution of electrophoretic mobilities, indicative of their surface properties.

In principle, CE-LIF is capable of monitoring multiple types of organelles simultaneously, which would be advantageous to track mitophagy by monitoring both mitochondria and autophagy organelles at once. To demonstrate this approach we labeled mitochondria with the mitochondria-specific dye Mitotracker Red and autophagosomes with anti-LC3 antibody conjugated to Dylight 488. The fluorophores were excited by an Argon ion laser at 488 nm and emit at 644 nm and 518 nm respectively. Separations were performed with running buffer containing sucrose (250 mM) and HEPES (10 mM) at pH 7.4. Dual labelled events were not detected above the false positive rate, which was expected because autophagy was not induced. In ongoing work we will perform pharmacological treatments (vinblastine to halt mitophagy and hydrogen peroxide to increase mitophagy) to confirm that dual-labeled events, indicative of mitochondria inside autophagosomes, are indeed associated with mitophagy.
The goal of this work is to develop a method for ambient laser ablation sampling for mass spectrometry and other bioanalytical techniques. An optical parametric oscillator laser system operating in the 3 µm wavelength region of the mid-infrared is used to ablate material from a cell or tissue sample that is drawn into a capillary by means of suction. The 1 mJ pulse energy beam is focused onto a tissue section on a microscope slide from the back in transmission mode or from the front in reflection mode. The transmission mode approach is most useful as a sample preparation method because the tissue slide can be held close to the suction capillary. The material is drawn into a peek capillary tube connected to a filter or impactor for collection. The collected material is brought into solution and further processed, for example by liquid chromatography separation, before being sent to an electrospray source for ionization. Alternatively, the solubilized material can be deposited on a target for matrix-assisted laser desorption ionization analysis. We have found that it is possible to transfer intact biomolecules such as peptides and proteins from thin tissue samples at a resolution of 130 µm. Both transmission and reflection modes methods have been tested. The ablation process greatly reduces the complexity of the tissue matrix. In both cases, digestion of captured protein material has been performed without any other extraction, detergent addition and/or purification step.

Acknowledgement: National Science Foundation (Grant No. CHE-1152106)

Keywords: Laser, Mass Spectrometry, Proteomics, Sample Preparation
Application Code: Bioanalytical
Methodology Code: Sampling and Sample Preparation
Sampling and Sample Prep of Bioanalytical Samples

A Novel, Synthetic Simplified Liquid Extraction (SLE) Sorbent that Alleviates the Downfalls of Traditional SLE

Supported Liquid Extraction (SLE) has become popular amongst labs that are looking for a fast and effective approach to sample preparation. Traditional SLE relies on diatomaceous earth (DE) to perform extractions. The process is simple and when formatted into a 96-well plate it can be automated for high throughput. However, as a natural material DE is known to exhibit lot-to-lot variances and supply of suitable low density porous material can be limited. In response to these challenges, a novel, synthetic SLE sorbent, Novum, has been developed.

Using protocols that are similar to DE SLE we illustrate the extraction efficiency of the Novum sorbent with common solvents (Ethyl Acetate (EtOAc), DCM, and MTBE). Extractions of NSAIDs, corticosteroids, as well as a panel of pain management drugs from human plasma are shown to be both fast (<5 minutes) and efficient (>80% recovery).

A major cleanup benefit of LLE and SLE is the elimination of phospholipids. Plasma samples were diluted 1:1 with water and were analyzed using two batches of Novum, and two batches of DE SLE. Using EtOAc as an elution solvent, phospholipids in the resulting extract were analyzed by LC/MS/MS using m/z 184-184 (as a measure of general cleanliness). Both lots of the synthetic SLE removed >99.9% of 5 major classes of phospholipids. The DE SLE, however, allows the partitioning of the phospholipids into the organic and shows a large variance between lots (up to 9 fold; as a function of variable loading capacity). Generally with DE SLE, solvents such as DCM or MTBE can completely remove phospholipids from an extract, but this can compromise recoveries of more polar analytes. This result shows that nearly complete elimination of phospholipids, and efficient extraction of both polar and non-polar analytes can be achieved using Novum and EtOAc as an extraction solvent.

Keywords: Biological Samples, Clinical/Toxicology, Liquid Chromatography/Mass Spectroscopy, Sample Preparation

Application Code: Bioanalytical

Methodology Code: Sampling and Sample Preparation
Selective Extraction of DNA Using Magnetic Ionic Liquids

DNA extraction represents a significant bottleneck in nucleic acid analysis. Traditionally, liquid-liquid extraction with phenol-chloroform was used for the purification of DNA from biological samples. Several adaptations to this method involving the addition of detergents to assist in the removal of proteins and polysaccharides have been made. However, the dependence of these protocols on organic solvents and often time-consuming centrifugation steps has resulted in the development of more environmentally benign techniques that are capable of high sample throughput. This talk will discuss the synthesis of hydrophobic magnetic ionic liquids (MILs) and their implementation as solvents for the rapid and efficient extraction of DNA from aqueous solution. The DNA-enriched MIL microdroplets were manipulated by application of a magnetic field. The MILs examined in this study exhibited unique DNA extraction capabilities when applied towards a variety of DNA samples and matrices. High extraction efficiencies were obtained for smaller single-stranded and double-stranded DNA using the benzyltrioctylammonium bromotrichloroferrate(III) MIL, while the dicationic 1,12-di(3-hexadecylbenzimidazolium) dodecane bis[(trifluoromethyl)sulfonyl]imide bromotrichloroferrate(III) MIL produced higher extraction efficiencies for larger DNA molecules. The recovery of DNA from the MIL extraction solvent and its implications in downstream analysis including polymerase chain reaction will be discussed.

Abstract Text

DNA extraction represents a significant bottleneck in nucleic acid analysis. Traditionally, liquid-liquid extraction with phenol-chloroform was used for the purification of DNA from biological samples. Several adaptations to this method involving the addition of detergents to assist in the removal of proteins and polysaccharides have been made. However, the dependence of these protocols on organic solvents and often time-consuming centrifugation steps has resulted in the development of more environmentally benign techniques that are capable of high sample throughput. This talk will discuss the synthesis of hydrophobic magnetic ionic liquids (MILs) and their implementation as solvents for the rapid and efficient extraction of DNA from aqueous solution. The DNA-enriched MIL microdroplets were manipulated by application of a magnetic field. The MILs examined in this study exhibited unique DNA extraction capabilities when applied towards a variety of DNA samples and matrices. High extraction efficiencies were obtained for smaller single-stranded and double-stranded DNA using the benzyltrioctylammonium bromotrichloroferrate(III) MIL, while the dicationic 1,12-di(3-hexadecylbenzimidazolium) dodecane bis[(trifluoromethyl)sulfonyl]imide bromotrichloroferrate(III) MIL produced higher extraction efficiencies for larger DNA molecules. The recovery of DNA from the MIL extraction solvent and its implications in downstream analysis including polymerase chain reaction will be discussed.

Keywords: Extraction, Nucleic Acids, Sample Preparation
Application Code: Bioanalytical
Methodology Code: Sampling and Sample Preparation
Hollow fiber based liquid phase micro-extraction (HF-LPME) technology has proven to be a unique technology that can be used for the analysis of compounds in many areas, especially in pharmaceuticals. For example, it can be used for the determination of log D, permeability and protein binding, etc. Specifically, we design and fabricated a 96 well plate with HF tubing sit right in the center of each well. One end of the HF was sealed while the other end was open and linked to the injected plastic 96 well plate base, which was specifically designed. On the side, another hole was made on the plastic plate for the delivery and extraction of liquid for the well. This 96 well plate based HF-LPME system is perfectly suited for the preparation of a few dozen microliter samples. At the same time, a proper auto-sampling device was made to accommodate this 96 well plate device making it ideal for the automation of isolation and sample clean-up in a high throughput fashion. With this new system, we were able to develop various analytical methods for the analysis of drugs in complex samples. We will report our recent progresses in the determination of log D and permeability of compounds. Plus, we will show our results for the determination of drug-protein interaction as well as the determination of drugs in complex biological samples. It is anticipated that this novel HT-HF-LPME technology will facilitate the drug discovery process with saving in both time and compounds/reagents.
Ablation by a picosecond infrared laser (PIRL) based on the principle of ultrafast desorption by impulsive vibrational excitation (DIVE) offers a novel tool to extract biological samples for analysis and diagnosis, while their integrity and function are preserved through the ablation. Featured by its wavelength at 3 µm and its pulse width of 300 ps, the PIRL ablation under DIVE conditions is a highly efficient process using water molecules to propel surrounding biological complexes into the gas phase. This well-confined ablation process minimizes shock wave damage and thermal degradation to biological samples. The PIRL ablation was applied to ferritin, recombinant Green Fluorescence Protein (rGFP), Tobacco Mosaic Virus (TMV), and Saccharomyces cerevisiae (S. cerevisiae) cells in frozen aqueous solutions or suspensions. Techniques including microscope imaging and analytical methods were used to demonstrate intact quaternary structure of ferritin, sustained fluorescence and absent degradation of rGFP, intact structure and preserved infectivity of TMV, and intact biological activities of S. cerevisiae cells collected from the ablation plumes. The collected ablated biological complexes are virtually identical to their controls and serve as valid samples for downstream analysis. The PIRL ablation presents a simple and direct method to extract biological entities with preservation of morphological and chemical properties, and biological activities. With no sample preparation required, great amount of time and effort can be spared using the PIRL ablation to extract biological entities for diagnosis, which is especially beneficial to disease control.

Grant AdG-2011-291630 from European Research Council is acknowledged for financial support.
Using Light for Bio-Analysis

Paper-Based Chemiluminescent Biosensing Platforms for Cotinine and Dichlorvos Detection

Paper-based biosensing platform or “lab on paper” has been exploited in analytical and clinical chemistry due to the fact that it is cheap, easy to use, disposable and environmental friendly. Chemiluminescence (CL) is a very sensitive detection method that requires no light source. We have combined two paper-based analytical methods with chemiluminescent detection; one is with immunoassay method for cotinine detection while the other is with paper chromatography for dichlorvos (DDV) detection. Laminated paper-based analytical devices (LPAD) with origami-enabled CL immunoassay have been developed for the detection of cotinine. Cutting/Lamination methods were used to fabricate LPAD and the simple origami step was adopted to eliminate possible reagent diffusion and flow during antibody immobilization steps and numerous washings. By incorporating luminol-based CL for detecting horseradish peroxidase-conjugated cotinine, cotinine in mouse serum was detected by using competitive immunoassay. The detection limit was determined to be 5 ng/mL. For the paper-based device for DDV detection, chromatographic separation for a sample was first accomplished in 12 minutes in a paper substrate (0.8×7.0 cm²). The interference patterns can be developed at different spatial locations and the detection area of DDV was cut to make a paper-based device for CL detection. By using luminol CL system, DDV can be detected with the detection limit of 3.6 ng mL⁻¹.

Abstract Title
Paper-Based Chemiluminescent Biosensing Platforms for Cotinine and Dichlorvos Detection

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Abstract Text
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Keywords: Analysis, Chemiluminescence, Luminescence
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Using Light for Bio-Analysis

Turn-On Two-Photon Fluorescence Sensor for Intracellular Imaging of Glutathione Using MnO2-Nanosheet-Modified Two-Photon Nanoparticles

Glutathione (GSH) serves vital cellular biological functions, and its abnormal levels are associated with many diseases. To better understand its physiological and pathological functions, efficient methods for monitoring of GSH in living systems are desired. Although quite a few small molecule-based and nanomaterial-based one photon fluorescence probes have been reported for GSH, two-photon (TP) probes, especially nanoprobes with good membrane-permeability, are more favorable for bioimaging applications, since TP fluorescence imaging can provide improved spatial localization and increased imaging depth. In this work, we for the first time reported a “turn-on” TP fluorescence nanoprobe for efficient detection of GSH in aqueous solutions, and TP excited fluorescence imaging of GSH in living cells and tissues. The nanoprobe consists of two-photon mesoporous silica nanoparticles (TP-MSNs) with a large TP excitation action cross-section ($\sigma$) value of 103 GM; and MnO2 nanosheets, which show intense and broad optical absorption and could act as efficient quenchers for TP fluorescence. In the sensing system, the negatively charged MnO2 nanosheets are adsorbed on the positively charged MSNs through electrostatic interaction, resulting in efficient quenching of their fluorescence, with very low background fluorescence observed. The addition of GSH could reduce MnO2 into Mn2+, lead to the decomposition of the MnO2 nanosheets, and thereby result in remarkable enhancement of both one photon and TP excited fluorescence of the nanosystem. The nanoprobe shows a highly sensitive response to GSH in aqueous solutions, with a detection limit of 200 nM achieved. It also exhibits a high selectivity toward GSH relative to other biomolecules and electrolytes, with good membrane-permeability and excellent biocompatibility. The nanoprobe was successfully applied in monitoring the change of the intracellular GSH in living cells and tissues via TP fluorescence imaging, demonstrating its value of practical application in biological systems.

Keywords: Bioanalytical, Biosensors, Imaging, Near Infrared
Application Code: Bioanalytical
Methodology Code: Sensors
Nanotechnology is a growing interest of many different scientific disciplines. Nanomaterials have secured an eminent position in many consumer products and medical applications. Better understanding of their effects on living organisms will enable us to rationally design more biocompatible nanomaterials for a wide variety of applications including design of smart drug delivery vehicles and probing of how brain functions. In this study, we investigated the mechanisms of effects of purified and well-characterized nanoparticles (NPs) on embryonic developments, specifically neurological development. To that end, we selected vital protein biomarkers that play important roles in neurological development, used transgenic embryos that express the protein biomarkers fused with fluorescence proteins as model organisms, and studied the effects of NPs on their functions using fluorescence microscopy and plasmonic spectroscopy. Updated results and applications will be discussed in this presentation.

Keywords: Bioanalytical, Biotechnology, Imaging, Nanotechnology
A plasmonic nanopipette based on a patch clamp pulled capillary will be presented. Plasmonic nanopipettes were fabricated using different methodologies, including direct deposition of different types of gold nanoparticles on nanopipette, focus ion beam milling of a gold film, glancing angle deposition, and polymer-assisted nanotemplating. The characterization of the optical properties of plasmonic nanopipettes with SEM, dark field microscopy, and Raman microscopy will be presented. The nanopipettes are envisioned as nanoprobes to monitor molecules with high spatial resolution and so, will allow a selective quantification of molecules (e.g., ATP and BMP-2) inside and nearby cells. Micro and nanoscale electrodes are used in several analytical techniques like patch clamp, and electrochemistry due to their non-destructive nature on the substrate justifying their use as a tool to study metabolites around living cells. However, specific analytes such as electroactive species are required. On the other hand, plasmonic materials used in biosensing allow quantification of multiple biomolecules due to their universal quantification behavior. By coupling these techniques, plasmonic nanopipettes allow localized quantification of molecules by the use of a SERS response based on a sandwich assay using a gold nanoparticle (AuNP) tagged with a Raman active reporter. Sensitivity, limit of detection (LOD), and reproducibility of the nanopipettes will also be optimized for the detection of IgG.

Keywords: Bioanalytical, Biosensors, Surface Enhanced Raman
Application Code: Bioanalytical
Methodology Code: Biospectroscopy
Using Light for Bio-Analysis

Anhydrobiotic Lipid Vesicles for On-Demand SPRi Analysis of Supported Bilayer Membrane Systems

Surface plasmon resonance (SPR) spectroscopy and SPR imaging (SPRi) have proven to be powerful analytical tools in the label-free analyses of molecular interactions, especially those involving supported bilayer membrane (SBM) systems. While the technology behind creating supported lipid bilayers on glassy surfaces is well developed, one current issue is the long-term stability of lipid membranes on the surface. Typically, lipid vesicles must be used soon after preparation, and once assembled into a bilayer must remain in aqueous conditions, thus limiting the large-scale production and application of such systems. Recently, a method that involves an anhydrobiotic sugar, trehalose, for preserving intact lipid vesicles has attracted attention. Once rehydrated, the trehalose dissolves into solution, and lipid vesicles immediately rupture and fuse into a continuous bilayer at the deposited surface. We have applied this technology toward creating lipid microarrays for SPRi analysis. The arrays can be generated in quantity and are viable for weeks. The resulting trehalose-facilitated bilayers were studied by SPRi and fluorescence recovery after photobleaching to confirm they retained the lateral mobility characteristic of typical SBMs. Finally, varying concentrations of the GM1 receptor were mixed with the lipids before desiccation and used for SPRi analyses of cholera toxin interactions to demonstrate the feasibility of long-term storage for high-throughput SBM substrates.

Keywords: Adsorption, Biosensors, Lipids, Membrane
Application Code: Bioanalytical
Methodology Code: Biospectroscopy
Formaldehyde (FA) is a hazardous chemical and found not only in industrial products but also in living bodies. It usually exits in mix gas, making it difficult to identify. Therefore it is highly demanded to develop a technique allowing sensitive and selective detection of FA vapor. Here we performed detection of gaseous FA transpired from food samples (Shiitake mushrooms) using a fiber-optic biochemical gas sensor (bio-sniffer). Owing to formaldehyde dehydrogenase (FALDH) employed in the bio-sniffer, nicotinamide adenine dinucleotide (NADH) is produced as a result of the enzymatic reaction with FA. Finally NADH is excited with UV light and emitted fluorescence is detected (Fig. 1). These enzyme and fluorescence lead to high selectivity and sensitivity for the FA measurement.

With standard gas generators, the LOD for FA vapor was determined as 1.1 ppb that is about 80-fold lower than WHO guideline for residential indoor air quality. When flowing the gas sampled from the dried mushrooms, the fluorescence intensity drastically increased (Fig. 2), indicating that the bio-sniffer is capable of detecting FA in complex gaseous samples as well as in pristine ones. In conclusion, the bio-sniffer can be a powerful tool for gas monitoring in the environment or gas analysis for medical diagnostics.

Acknowledgements:
This work was supported by Japan Society for the promotion of Science (JSPS) Grant-in-Aid for Scientific Research System, Japan Science and Technology Agency (JST) and Ministry of Education, Culture, Sports, Science and Technology (MEXT) Special Funds for Education and Research “Advanced Research Program in Neo-Biology”.

Keywords: Bioanalytical, Fiber Optics, Gas
Application Code: Bioanalytical
Methodology Code: Sensors
Accurate detection of protein levels in biological samples is critical to understanding various biological processes and disease states. However, the direct detection of protein levels in natural samples is inherently challenging due to the extreme complexity of the sample matrix. One way to get around this limitation is to use an array of cross-reactive sensor elements which respond differently to different proteins. Herein, we report a fluorescence sensor array fabricated by using a Group of Uniform Materials Based on Organic Salts (GUMBOS) for highly sensitive detection and discrimination of proteins. These GUMBOS were prepared by pairing a derivative of naphthalenesulfonate anion with five different phosphonium cations. The GUMBOS and proteins exhibited efficient and discrete Förster Resonance Energy Transfer (FRET) between them. The FRET signal patterns of proteins generated from GUMBOS sensor array were statistically analyzed by using principal component analysis and linear discriminant analysis in order to accurately identify proteins. Overall, this array is very promising for facile, inexpensive and label-free detection and discrimination of proteins with a high degree of accuracy.
Recent studies reveal that 47% of all the marketed small molecules drugs act on enzymes. Indeed, many drugs based on enzyme inhibition have been commercialized, demonstrating the importance of enzyme inhibitors. Diagnosis of enzyme inhibition and determination of inhibition constant are often found in papers dealing with bioassays and biosensors. Although the huge number of papers published in this field, analyzing all reaction time-course of progress curve of enzyme inhibition is scarce although it contains additional information about the properties of the enzyme.

In this work, the advantages of a progress curve analysis versus an initial rate approach will be highlighted. Inhibitor concentration was determined by means of “half time reaction” estimated by progress curve, avoiding the use of end-point and initial rate approaches. Simplified equations and a novel graphical method are proposed, valid for all types of reversible inhibition, for the determination of the inhibitor concentration with an extended linear range and for the estimation of the inhibition constant $K_i$. Practical examples of inhibition of catalase by cyanide, cholinesterase by fluoride, peroxidase by sulphide and chromium and carbonic anhydrase by sulfonamide are reported. Limitations of the proposed method and results of real sample analysis will be discussed.

Acknowledgements:
The authors gratefully acknowledge the financial support of the European Commission Proposal No FP7 OCEAN-2013-613844 and NATO Science for Peace project SFP.984173.
The rapid spate of development of many industries leads to wastes containing heavy metals being directly or indirectly discharged into the environment. This study employed the adsorption of cadmium (II), cobalt (II), and nickel (II) on corncob, an agricultural waste has been studied in batch mode at room temperature using flame atomic absorption spectroscopy for metal estimation. The study investigated the influences of adsorbent dosage, solution pH, contact time, and initial metal concentration on the removal of metal ions. The Langmuir and Freundlich isotherm models were used to fit the equilibrium biosorption data. The reaction kinetics of the cadmium (II), cobalt (II) and nickel (II) removal from the aqueous solution were identified and correlated to the pseudo first- and second-order kinetic models. The results show an increase in adsorption by metal ions studied with an increase in adsorbent dosage. Metal uptake showed a pH-dependent profile with optimum at pH values 4.0 for Cd (II) as well as Ni (II), and 8.0 for Co (II) ions. The Freundlich adsorption isotherms model fitted the experimental data best with the regression coefficient ranging between 0.837 and 0.989 for the metal ions. A kinetic study of the adsorption process showed that adsorption of cadmium (II), cobalt (II), and nickel (II) on corncob was observed to follow closely to the pseudo-second–order. It was concluded that corncob exhibited rapid adsorption capabilities and high efficiency in removal of cadmium (II), cobalt (II) and nickel (II) ions from aqueous solution.

Keywords: Adsorption, Agricultural, Analysis, Metals
Application Code: Bioanalytical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Bioanalytical Samples Using Separation Techniques

An Integrated Platform of LC-MS, HPAEC-PAD, and Bioinformatics for the Carbohydrate Profiling of Therapeutic Glycoproteins

In the biopharmaceutical industry, there is an increasing interest in monitoring the effect that carbohydrate components have on the structural and functional roles of a therapeutic glycoprotein. Glycosylation patterns of recombinant glycoproteins are influenced by factors including the expression and growth conditions. These patterns affect the biological activity of proteins, such as the immunogenicity and receptor binding, which may subsequently affect the efficacy and safety of the final product. The goal is to determine the carbohydrate composition of immunoglobulin G (IgG) monoclonal antibody, a model glycoprotein, produced in miniature bioreactors compared to bench-scale models. Work will be performed using the complimentary techniques of HPAEC-PAD and LC-MS for profiling and characterization.

HPAEC-PAD glycan profile analysis used enzymatically cleaved glycans from IgG3 by PNGase F and isolated with carbon top-tips. The monosaccharide sequence of glycans will be characterized via LC-MS fragmentation studies using a carbohydrate mixed-mode column.

Three major glycan structures were found and characterized as asialo-fucosylated, asialo-monogalacto-fucosylated, and asialo-agalacto-fucosylated biantennary glycans. Mass spectrometry sequencing experiments were performed for insulin and streptokinase using top-down and bottom-up approaches, and 100 percent sequence coverage was achieved through the bottom-up method. Full intact protein identification was verified with the top-down approach. These methods will be applied to IgG3. Although the mini-HTB is able to overcome the previously mentioned issues, it introduces the need to analyze relatively small volumes of sample. The methods of LC-MS and HPAEC-PAD offer the needed sensitivity to work with small sample quantities.

*Funding: DARPA-BAA-12-37 - Biologically-Derived Medicines on Demand

Keywords: Bioanalytical, Carbohydrates, Electrochemistry, Liquid Chromatography/Mass Spectroscopy
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Bioanalytical Samples Using Separation Techniques

Analysis of Polysorbate 80 in Protein Formulations Using 2D LCMS

Polysorbate 80 is commonly used for biotherapeutic products to prevent aggregation and surface adsorption, as well as to increase the solubility of biotherapeutic compounds. A reliable method to quantitate and characterize polysorbates is required to evaluate the quality and stability of biotherapeutic products. Several methods for polysorbates analysis have been reported, but most of them require time-consuming sample pretreatment such as derivatization and alkaline hydrolysis because polysorbates lack sufficient chromophores. Those methods also require an additional step to remove the biotherapeutic compounds. Here we report a simple and reliable method for the quantitation and characterization of polysorbates in biotherapeutic products using two-dimensional HPLC.

A model sample was prepared for this study. The model sample contained 20 mg/mL of human IgG and 0.1 mg/mL of polysorbate 80 in 10 mmol/L phosphate buffer pH 6.8. This sample was injected on a Shimadzu Co-sense for BA two-dimensional HPLC system coupled with a Shimadzu LCMS-2020 single quadrupole mass spectrometer. The IgG was separated from the polysorbates which were retained on a 5mm x 2.0mm trap column. The polysorbates were then introduced to an analytical column by valve switching. The polysorbate 80 was detected by the mass spectrometer.

The trap column, Shim-pack MAYI-ODS, successfully separated the analytes from the IgG. Polysorbates are complex mixtures of polymeric compounds, so many peaks were present on the total ion chromatogram. A selected ion monitoring mode and a short analytical column were used for quantitation and a total ion chromatogram and long analytical column were used for characterization.

Keywords: Bioanalytical, Biopharmaceutical, HPLC, Liquid Chromatography/Mass Spectroscopy
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Bioanalytical Samples Using Separation Techniques

Using Fluorescence and Mass Spectrometry to Determine Glycation Sites of Human Serum Albumin

The non-enzymatic glycation of blood and tissue proteins has been shown to lead to the formation of Advanced Glycation Endproducts (AGEs); factors contributing to many of the chronic complications of diabetes such as renal failure, atherosclerosis and cataract formation. The clinical and medical significance of AGEs has promoted interest in several areas including methods to characterize AGEs and parameters/reagents that influence the glycation process. The objective of this study was twofold: 1) using fluorescence spectroscopy to characterize the side chain modification of human serum albumin glycated by dihydroxyacetone (DHA), a commonly used tanning reagent in cosmetic products, 2) to identify by peptide mapping the amino acid sites in albumin vulnerable to DHA glycation. Fluorescence studies showed that DHA was a potent glycator of albumin, extensively modifying the protein’s lysine and arginine residues. Peptide mapping of early glycation products identified lysine 201 as the major site of glycation with MALDI-TOF studies demonstrating an average of 5 DHA molecules bound to the protein. The reactivity of DHA with lysyl residue 201 was interesting because this site was never reported as a principle site of glycation in HSA.

Fluorescence, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry

Weixi Liu
University of Rhode Island

Joel A. Dain, Leslie Frost, Menashi A. Cohenford

Date: Thursday, March 12, 2015 - Morning
Time: 
Room: Exposition Floor, Hall F, Aisles 390

Fluorescence, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry

Bioanalytical

Liquid Chromatography/Mass Spectrometry
Bioanalytical Samples Using Separation Techniques

An Improved Chromatographic Method for Arsenic Speciation in Urine

Arsenic speciation in urine is one of the most common HPLC-ICP-MS analyses due to the importance of distinguishing toxic and non-toxic species. Most work has focused on anion exchange separation of the various arsenic species. Although successful, this separation scheme suffers from several drawbacks, the most notable being the difficulty in measuring low-levels of inorganic arsenic (specifically trivalent arsenic, AsIII) in the presence of high levels of non-toxic organo-arsenic compounds (specifically arsenobetaine, AsB). While inorganic arsenic is present in low-levels in a variety of foods, AsB enters the body through consumption of marine organisms, which metabolize arsenic to form AsB.

With most anion exchange separation methods, AsB elutes just prior to trivalent arsenic. Since AsB levels are usually much higher than AsIII, the broad peak with long tail from high levels of AsB can obscure the presence of AsIII.

This work will explore an alternate separation scheme which changes the order in which the arsenic species elute, thus allowing low levels of AsIII and AsV to be determined in the presence of high levels of AsB.

Keywords: HPLC, ICP-MS, Speciation
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
The Maillard Reaction is a complex series of reactions between an amino acid and a reducing sugar that has important physiological implications, particularly for diabetes mellitus. In this presentation, we will describe the application of a sensitive and selective analytical method that we have developed to study the kinetics of the initial stages of the Maillard Reaction. The method is based upon sample pre-treatment by sequential injection (SI) prior to detection by low-field nuclear magnetic resonance spectroscopy (NMR). The SI-NMR method improves the sensitivity and selectivity of the picoSpin™ detector by effectively pre-concentrating the sample before the sample zone enters the flow cell of the spectrometer. The initial steps in the reaction between alpha-dicarbonyl compounds (e.g., glyoxal and methyl glyoxal) and primary amines (e.g., methylvamine and lysine) were monitored on-line with SI-NMR. This approach allowed for the determination of the rate constants, reaction orders, and structures of the intermediates that were formed. A mechanism for the production of the Amadori product will also be proposed.
An Automated Dual Stage Solid Phase Extraction Procedure for 15 F2t-isoprostane (8-isoprostaglandin2a) from BSA as Lipid Markers of Oxidative Stress

F2-isoprostanes are lipid markers of oxidative stress in animal host systems. F2-isoprostanes have been identified as biomarkers of cardiovascular disease, pulmonary disease, neurological disorders and various conditions related to the health and function of the kidneys and liver. Strategies to measure these analytes are typically laborious multi-step procedures that are plagued with low recovery sample preparation strategies leading to poor method precision. In a broader scope, targeting endogenous lipids as analytes of interest is challenging since modern solid phase extraction procedures (SPE) typically aim to remove lipids in favor of targeting small molecules. For these reasons, method optimization strategies are required to improve the efficacy of current testing protocols. In this work, the automation of an existing SPE procedure was implemented. A dual stage cleanup strategy was employed. Automation was achieved using a RapidTrace+ system with data acquisition software. The SPE platform was 3 mL cartridges supporting 500 mg sorbent beds. Detection of the target analytes was optimized using a Shimadzu gas chromatography system (QP-2010) coupled to a quadrupole mass selective detector. The GC column was an Rtx-5 30 meter, 0.32 mm ID, 0.25 μm df, Restek Corp, Bellefonte, PA. The ion source was operated in the negative ion chemical ionization mode. It was determined that there were several observed advantages to this approach vs offline manual preparation including laboratory efficiency and figures of merit describing data quality. It is anticipated that this report will have impact in labs that provide testing platforms for population studies focused on lipids as markers of oxidative stress. This automation platform demonstrates an improvement in lab efficiency and data quality. Preliminary data for this study was generated at the University of Kentucky.

Keywords: Bioanalytical, Clinical/Toxicology, GC-MS, Solid Phase Extraction
Application Code: Bioanalytical
Methodology Code: Sampling and Sample Preparation
Imaging mass spectrometry (IMS) has been used by a variety of scientists to address research questions of chemical and biochemical significance. In terms of data processing, traditional two-dimensional heat maps of single ions lack sophisticated analytical detail, yet manual analysis of multiple peaks across hundreds of pixels within an entire image is time-consuming, tedious, and subjective. Here, various chemometric methods are used to analyze datasets obtained by matrix-assisted laser desorption/ionization (MALDI) IMS of a three-dimensional cell culture system. HCT-116 colon carcinoma multicellular spheroids are an excellent [i]in vitro[/i] model that mimic the three dimensional morphology and cellular heterogeneity of tumors [i]in vivo[/i]. This particular dataset is especially challenging to process because all cells within the image are genetically identical which can sometimes result in only subtle differences in protein expression (and thus the mass spectral profiles) within the image. First, principal component analysis (PCA) was used to isolate relevant pixels within the recorded MALDI IMS image. The data was further processed prior to a second round of PCA whereby mass spectral features were identified within the image. Next, clustering methods were used to identify subpopulations of pixels within the image that exhibited similarities in spectral content. Finally, discriminant analysis was used to identify spectral features present in spatially heterogeneous locations within the image. These exploratory data analysis tools allow for the isolation and detection of biochemical phenomena within IMS datasets in an easy, rapid, and unbiased manner.
Bioanalytical Samples Using Separation Techniques

Effects of SDS and DTT in Protein Determination Using the 660 nm Protein Assay

As a simple and rapid single reagent colorimetric assay, the Pierce® 660 nm Protein Assay is one of the commonly used methods for protein determination. Certain substances, such as detergents, reducing agents, and buffers may interfere with the Pierce® 660 nm Protein Assay. In our study, we analyzed the interfering effects of the detergent Sodium Dodecyl Sulfate (SDS) and the reducing agent Dithiothreitol (DTT) present in samples for protein determination using the Pierce® 660 nm Protein Assay microtiter format. We also explored the correlations of the measured protein concentrations with these interfering substance concentrations to target protein values. Multiple concentration levels of Bovine Serum Albumin (BSA) were prepared in matrices containing various levels of SDS and DTT. The results of the analyses demonstrated that the measured protein concentration was decreased with an increase in SDS concentration, and the percent decrease was independent from the protein concentration of the samples. The effects of both SDS and DTT presence in the sample matrices analyzed for protein concentration were explored with and without the usage of the Pierce® Ionic Detergent Compatible Reagent (IDCR).

Keywords: Analysis, Bioanalytical, Protein, Spectrophotometry

Abstract Title
Effects of SDS and DTT in Protein Determination Using the 660 nm Protein Assay

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Abstract Text
As a simple and rapid single reagent colorimetric assay, the Pierce® 660 nm Protein Assay is one of the commonly used methods for protein determination. Certain substances, such as detergents, reducing agents, and buffers may interfere with the Pierce® 660 nm Protein Assay. In our study, we analyzed the interfering effects of the detergent Sodium Dodecyl Sulfate (SDS) and the reducing agent Dithiothreitol (DTT) present in samples for protein determination using the Pierce® 660 nm Protein Assay microtiter format. We also explored the correlations of the measured protein concentrations with these interfering substance concentrations to target protein values. Multiple concentration levels of Bovine Serum Albumin (BSA) were prepared in matrices containing various levels of SDS and DTT. The results of the analyses demonstrated that the measured protein concentration was decreased with an increase in SDS concentration, and the percent decrease was independent from the protein concentration of the samples. The effects of both SDS and DTT presence in the sample matrices analyzed for protein concentration were explored with and without the usage of the Pierce® Ionic Detergent Compatible Reagent (IDCR).
Bioanalytical Samples Using Separation Techniques
Coupling Advanced Detection Techniques to Size Exclusion Chromatography

Size Exclusion Chromatography is a well-established technique for biomolecule analysis, particularly for quantitation of monomer, dimer and higher aggregates in biotherapeutic manufacture. Such proteins form aggregates under stress conditions and can arise at many stages of manufacture – during isolation, purification, concentration or storage. Conventional silica-based stationary phases provide good resolution of such molecules, separating the molecules based on their size in solution. In order to overcome non-specific interactions that occur between basic sites on the proteins and acidic silanol groups of the silica stationary phase, the mobile phase includes a high concentration of salt (0.3 – 0.5M is not atypical). Such mobile phase conditions are satisfactory for concentration detectors such as UV or DAD, however this prevents more sophisticated detectors such as MS being used.

In this poster, we will show results obtained on an optimized size exclusion chromatography column that overcomes some of these limitations. Carefully controlled pore size distribution ensures the column is targeted specifically towards the molecular weight range of interest, and the particle size is designed to provide high separation efficiency. Furthermore the stationary phase is bonded to minimize secondary interactions and enables separations to be carried out at low salt concentrations and low ionic strength. We will illustrate some of the benefits of these new columns, particularly when coupled to advanced detectors and instrumentation.

Keywords: Bioanalytical, HPLC Columns, Liquid Chromatography, Pharmaceutical
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Gangliosides have been identified as potential biomarkers in neurological disorders. They are expressed highly in the central nervous system and play a major role in regulating vital cellular processes including: intracellular signalling, molecular recognition and cell adhesion. There is a growing interest in the spatial distribution and expression of these molecules in the brain. We imaged the localization of gangliosides and other bio-active lipids in C57BL/6NJ mouse tissue with MALDI-TOFMS. Preliminary complementary images of small molecule metabolites in the brain were produced from adjacent coronal mouse brain sections. We hope to expand our study to mine lipid and metabolite data for dynamic relationships and expression patterns within the brain.

Keywords: Bioanalytical, Lipids, Mass Spectrometry, Time of Flight MS
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Bioanalytical Samples Using Separation Techniques

Evaluation of Porous Layer Thickness of Core Shell Particle for Separation of Proteins

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Abstract Text

The feature of superficially porous (core shell) particle used as a highly efficient material is existence of a core, a thin porous layer and narrow particle size distribution, which lead to higher efficiency than totally porous particle. Recently a core shell particle with wide pore for biomacromolecular separations has developed by a few manufacturers. It has been said that thin porous layer of core shell particle have an advantage for separation of large molecules such proteins because a diffusion coefficient becomes small to proportional to a molecular weight and a mass transfer speed also decreases. In this paper, thickness of porous layer of core shell particle was evaluated to separate proteins. 2 kinds of thickness of porous layer such as 0.2 mm and 0.5 mm thickness were applied for separation of standard protein samples. On fast separation, 0.2 mm of porous layer showed sharper peaks than 0.5 mm of porous layer. However at 80 degree Celsius and using 60 min gradient time program, 0.5 mm of porous layer showed much sharper peaks than 0.2 mm of porous layer. It was considered that 0.5 mm of porous layer had the a wider specific surface area than 0.2 mm of porous layer and this wider specific surface area leaded separation efficiency concerning the partition interaction on the stationary phase to be large.

Keywords:  
Bioanalytical, HPLC Columns, Protein

Application Code:  
Bioanalytical

Methodology Code:  
Liquid Chromatography
Fleurya aestuans is used in alternative as a uterotonic agent to shorten labor and hasten the detachment of the placenta. It is also used as an antimicrobial, astringent, and in the management of menopausal and liver disorders and burns. The purpose of the study is to identify constituents of the essential oil from the plant which may exhibit these bioactivities. 

The essential oil was extracted by hydrodistillation using a Clevenger apparatus and collected into hexane in two modes, namely: a continuous collection for four hours and hourly collection over the same period. The essential oil samples were analyzed on Agilent GC 7890A fitted with an HP 5 MS column 30 m x 0.320 mm x 0.25 µm using a temperature program of 80 deg.C (2min) increased at 5 deg./min to 120 deg. (2min) further increased at 3.5 deg./min to 240 deg. (15min) using helium as carrier gas. Mass spectrometry of the constituents was on Agilent MSD 5975C. The major constituents in the samples are 2-pentadecanone, 6,10,14-trimethyl (25.6-48.3%) and phytol (18.6-56.1%). Minor constituents include tetradecanal, dibutyl phthalate, docosane and tetracosane.

Phytol has been reported to exhibit anti-inflammatory and anti-tumor properties and found effective in suppression of arthritis, lowering of serum levels of triglycerides and management of diabetes, obesity and cardiovascular diseases. Thus phytol may promote wellness in pregnant women especially those who may develop diabetes or hypertension, hence the description of the plant as being a pregnancy booster. It may also shrink hemorrhoids. Isophytol has been reported damage fetus and may expel placenta or induce labor. Long chain alkanes may produce soothing effects in burns while 2-pentadecanone, 6,10,14-trimethyl and tetradecanal may function as astringent. The constituents of the essential oil samples thus have bioactivity related to some of the medicinal uses of the plant.

Funding is by the University and the authors.

Keywords: Analysis, Extraction, Flavor/Essential Oil, GC-MS
Application Code: Bioanalytical
Methodology Code: Gas Chromatography/Mass Spectrometry
Capillary electrophoresis is a powerful separation method due to its high efficiencies and its ability to analyze nanoliter volume samples, making it ideal to couple with microdialysis sampling. Electrochemical detection (EC) offers several advantages over other detection methods, notably its subnanomolar detection limits, and biological analytes of interest are already electrochemically active. However, capillary electrophoresis produces high separation currents that can greatly affect the EC detector. A decoupler is a solution that provides a break in the capillary, which is then covered by a semi-permeable material that will allow only current to escape the capillary. This permits the separation current to exit the capillary and into ground, while analytes remain in the capillary and are pushed towards the working electrode with the assistance of electrophoretic flow. A decoupler mechanism, previously designed in our lab, was implemented where a capillary was ablated with several holes using a CO2 laser, and cellulose acetate was used to provide a membrane. This design can dissipate larger amounts of current compared to previous designs, while providing improved structural support. Thiols and disulfides are biomarkers of oxidative stress. Thiols react with reactive oxygen species and are converted into disulfides. The ratio of thiols and disulfides can provide information on the amount of damage to DNA, proteins, and/or lipids. In particular, N-acetylcysteine, cysteine, cystine, homocysteine, homocystine, glutathione, and glutathione disulfides were detected in a parallel-opposed dual Au/Hg electrode design. This design allows for the simultaneous or individual detection of thiols or disulfides.

Keywords: Capillary Electrophoresis, Chemically Modified Electrodes, Electrochemistry, Method Development
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Many laboratories use GC/MS (Gas Chromatography/Mass Spectrometry) and GC/MS/MS for the analysis of steroids and hormones to detect and monitor health disorders. While LC/MS/MS (Liquid Chromatography/MS/MS) performed with triple quadrupole instruments is being used more frequently, it suffers from being a target analysis technique. Only the target analytes are detected, and it is not possible to retrospectively query the data for unexpected steroids, precursors, or derivatives in novel metabolomes. GC/MS is non-selective, providing a comprehensive view of all steroids in the metabolome for non-targeted steroid profiling. GC/MS also allows for spectral searching of unknown compounds against large libraries of reference spectra. LC introduction has the advantage that derivatization is generally not required, whereas GC typically requires it because of thermolability at the high analyte boiling points.

The technique of Cold EI GC/MS allows the introduction of high flow rates of carrier gas into the MS interface. Using relatively short GC columns, the high flow rates reduce the temperature required to elute the analytes and minimize decomposition. The Cold EI axial flow-through ion source and supersonic jet expansion reduces ion source decomposition compared with conventional closed EI source designs by eliminating analyte contact with hot source walls. Cold EI also enhances the molecular ion intensities, improving technique specificity. When used in a novel q-TOF configuration the enhanced molecular ion also increases MS/MS quantitative sensitivity, selectivity, and information content.

This paper will discuss the application of Cold EI GC/MS and GC/MS/MS to the analysis of underivatized steroids, simplifying and cost-reducing laboratory workflows.

**Keywords:** Bioanalytical, Derivatization, Gas Chromatography/Mass Spectrometry, Metabolomics

**Application Code:** Bioanalytical

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Bioanalytical Samples Using Separation Techniques

Analysis of Subtle Changes in Biological Systems Through Use of High Resolution, High Accuracy Vanquish UHPLC Generated Libraries with a Q-Exactive HF Mass Spectrometer

Protein diversity in biological systems is quite large, but short term (<1 hour) changes due to a particular stimulus will cause only subtle changes in very specific protein expression levels. Excessive sample-handling/processing methods often add significant noise to overall experimental results. Here, we use a combination of a simple sample preparation technique, the extremely reproducible Thermo Scientific™ Dionex™ Vanquish™ UHPLC system, and a robust mass spectrometer platform to afford researchers the ability to use massive protein/peptide libraries for quantification of several thousand proteins.

An in vitro model was created using live gram positive and gram negative bacteria spiked into whole blood and after which both the plasma and the Peripheral Blood Mononuclear Cells (PBMC) are extracted. The proteins were digested and a portion of each was used to make pools (plasma and PBMC protein digest pool). These pools were fractionated using an orthogonal high pH reverse phase gradient. The library was generated by spiking retention time QC standard peptides into each fraction, and each fraction was injected onto the identical LC method to be used for quantification, with additional MS time dedicated to sequencing. The resulting data was then searched using Thermo Scientific™ Proteome Discoverer™ software, and the results were imported into Thermo Scientific™ PinPoint™. Individual sample analyses were then compared against the database, with <5 s retention time reproducibly. If a peptide was not sequenced in a data dependent acquisition strategy, or if its mass overlapped with another peptide, accurate quantification/assignment was still possible.

In this case, the accurate quantification of several hundreds of proteins in plasma, with sub 7% CV with an 850 (1%fdr) library, is reported. Furthermore, accurate quantification of several thousand PBMC peptides, with sub 7% CV, with a 6600 protein library was achieved.

Keywords: Biological Samples, Data Mining, HPLC, Liquid Chromatography/Mass Spectroscopy

Application Code: Bioanalytical

Methodology Code: Liquid Chromatography/Mass Spectrometry
Dietary copper deficiency is associated with a variety of signs/symptoms of the metabolic syndrome, including hyperlipidemia and fatty liver. In order to investigate whether copper deficiency plays a role in fructose-induced fatty liver, male weanling Sprague-Dawley rats were housed in stainless steel cages in a temperature and humidity controlled room with a 12:12 h light–dark cycle. All rats were split into four groups. Each group of rats was fed either an adequate copper or marginal copper deficient diet. At the same time, distilled water or distilled water containing 30% fructose (w/v) was given as free access drinking for 4 weeks. Fructose enriched drinking water was changed twice a week. At the end of the experiment, all the animals were killed under anesthesia with pentobarbital. Colon stools were collected and snap-frozen with liquid nitrogen for metabolomics study.

Metabolites were extracted using a mixture solvent (methanol and water, v:v=8:2). The extracted metabolites from each fecal sample were derivatized using N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) mixed with 1% tert-butyldimethylchlorosilane (TBDMSCI). The derivatized samples were analyzed on a LECO Pegasus 4D GC×GC-TOF MS instrument. Experimental data were processed by ChromaTOF software for peak picking and compound identification, followed by MetPP software for retention index matching, cross sample peak alignment, normalization and statistical significance tests.

Preliminary analysis shows a significant metabolite profile difference between sample groups. A list of compounds with significant abundance changes was identified via pairwise two-tail t-test with sample permutation. The fecal metabolites profile reveals novel mechanism underlying marginal copper high fructose diet-induced NAFLD.

Keywords: Bioanalytical, GC-MS, Identification, Metabolomics
Application Code: Bioanalytical
Methodology Code: Gas Chromatography/Mass Spectrometry
Many purified protein materials used as active components in clinical diagnostic reagent test kits contain readily oxidizable moieties that can cause storage instability of potency due to crosslink-driven oligomerization, peptide bond cleavage or alteration of binding surfaces. In order to assure storage stability for this type of protein reagent, oxidant-scavenging reducing agents are typically added to the solution matrix. Reduced-thiol molecules such as dithiothreitol (DTT), glutathione (GSH), N-acetylcysteine (NAC) and 2-mercaptoethanol (BME) are often employed to fill this antioxidant role. Over the storage and eventual processing times for the protein reagent, the reducing agent in the matrix is presumed to be consumed in reactions during protection from naturally occurring oxidizing species. To design an effective solution composition for the expected storage lifetime of this type of protein reagent, an analytical method for measurement and monitoring of remaining active-reducing thiol matrix is needed. Although the classic Ellman’s method is useful for measurement of total active thiol content in solutions, we also encounter a frequent need to quantify specific thiol-containing molecules in matrices with more than one of these analytes present. We have optimized and characterized a RP-HPLC method using a fluorescent thiol-reactive label for measurement of concentrations for several specific reducing thiol reagents typically used in protein storage solutions. Analytical performance characteristics for the optimized method will be discussed along with example applications of the method to the assessment of reduced-thiol stability in protein storage diluent formulations.

## Keywords:
- Analysis
- Bioanalytical
- Fluorescence
- HPLC

## Application Code:
- Bioanalytical

## Methodology Code:
- Liquid Chromatography
Peptide mapping methods have historically been lengthy, time-consuming methods requiring large amounts of solvent. Modern instrumentation and smaller particle size stationary phases now allow for these methods to be scaled down; improving throughput, saving solvent, and increasing scientists’ overall productivity. In this poster we investigate the use of YMC-Triart C18 1.9um hybrid and YMC Meteoric Core C18 2.7um core-shell materials for improving peptide mapping methods.

Keywords: Bioanalytical, Biopharmaceutical, HPLC, Liquid Chromatography
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Branched chain amino acids (BCAAs) – valine, leucine and isoleucine – have been found to be involved in nutritional signaling, synthesis of proteins and control of appetite. Circulating BCAAs are found elevated in obese individuals and have been linked with metabolic disorders such as diabetes. In vivo experiments provide the unique opportunity to examine overall dynamics of BCAAs within different types of tissue as they function within interworking bodily systems. Using an online microdialysis-capillary electrophoresis instrument allows for in vivo BCAA dynamics to be monitored in response to external stimuli such as glucose, insulin and artificial sweeteners. Microdialysis probes allow for continuous analysis with minimal damage to the surrounding tissue upon probe implementation. High speed capillary electrophoresis pairs well with microdialysis sampling by overcoming the limitation of small sample volumes and provides full separation of amino acids of interest within 30 seconds. Further investigation into the dynamics of BCAAs as they occur in near real time will provide a more complete understanding of their detailed role in metabolism and how their function is correlated with adipose tissue and obesity.
Determination of Nitrite Using Captopril by UV Spectrophotometry and Flow Injection Analysis

The determination of nitrite is important for quality control of food, particularly processed meats. We have shown that nitrite rapidly nitrosylates the R-SH compound captopril under acidic conditions to produce the R-SNO nitroso derivative with absorption bands at ~333 nm and ~546 nm. Maximum absorbance at 333 nm is reached in ~3 min at pH 1 or ~40 min at pH 2 when using equimolar nitrite and captopril concentrations at room temperature, indicating a molar absorptivity of about 1080. The optimum reaction time at pH 2 can be decreased to about 20 min when the temperature is raised to either 40 or 50 [sup]°C. In addition, we have incorporated flow injection analysis (FIA) using a pump to propel the strong acid carrier (either sulfuric or perchloric acid) through two HPLC injection valves, a mixing tee, and a HPLC detector. Equal volumes of captopril solution at pH 1 and nitrite solution at moderate pH are simultaneously injected into the carrier stream and combine at the mixing tee. On-line absorbance measurements are performed using MeasureNet. Optimization of the flow rate showed the best response at 0.2 mL/min. Figures of merit including limit of detection, limit of quantitation, linear dynamic range and analysis time for the optimized flow system will be compared to the results of cuvette-based UV spectrophotometry. The effect of ionic strength and potentially interfering species on the absorbance signal will also be discussed.

Keywords: Flow Injection Analysis, UV-VIS Absorbance/Luminescence
Application Code: Food Science
Methodology Code: UV/VIS
The most important factor determining the shelf life of olive oil is its oxidative stability: lipid oxidation not only produces rancid odours, unpleasant flavours and discoloration, but it can also decrease the nutritional quality and safety of olive oil. Red chili pepper is widely used in its ground form, as natural antioxidant ingredient to retard or prevent lipid oxidation, to preserve the quality and to extend the shelf-life of food products, thanks to its high content of carotenoids, polyphenolic compounds and capsaicinoids, responsible for its characteristic hot taste.

In this work, the oxidative stability of the oil with chili pepper powder was investigated by accelerating the oxidation process using Oxitest reactor (VELP Scientifica; Italy), based on the use of high temperature and over-pressure of oxygen. The addition of few grams of red chili pepper powder to three edible oils (olive, extra-virgin olive, and seed oil), increases their stability since the beginning and during storage. The investigation was also extended to the use of three pepper powders with different level of piquancy. Among the three pepper powders tested, the strongest effect was recorded with the less piquant one. Therefore, the antioxidant effect could not be attributed to the capsaicinoids, responsible for piquancy, but to the presence of other antioxidant phenolics. As the latest evaluation, a dose-effect relation was studied among different amounts of pepper powder and the stability of olive oil and extra-virgin olive oil. An evident linear dependence was found in olive oil, related to the higher oxidative stability of extra-virgin olive oil, due to its high content of natural polyphenols.

This study suggests interesting perspectives of the extension of shelf life using olive oil added with mild red chili pepper powder in the food industry, as canned food in oil production.

Keywords: Analysis, Food Science, High Temperature, Lipids
Application Code: Food Science
Methodology Code: Other
Basmati rice is a naturally fragrant variety of long grain rice mainly cultivated in South Asia. Its typical flavor is due to the presence of 2-acetyl-1-pyrroline. Difficulty in differentiating genuine basmati from other types of rice and the significant price difference between them has led fraudulent traders to adulterate basmati rice with cheaper crossbred basmati varieties or long-grain non-basmati varieties.

This study describes the analysis of several batches of so-called Basmati rice with an electronic nose. The objective is to quantify the concentration in 2-acetyl-1-pyrroline, an indicator of Basmati rice quality, and to characterize the overall aroma. Ten batches of Basmati rice (R01 to R10) from different suppliers were evaluated with HERACLES electronic nose, based on two-column fast gas chromatography. Before headspace analysis, rice is cooked by placing a fixed mass of rice in a vial containing an aqueous solution of saturated NaCl and heating. 2-acetyl-1-pyrroline is detected on the chromatograms at a retention time of around 71s on MXT-5 and around 76s on MXT-1701 column. Basmati rice usually contains 2-acetyl-1-pyrroline at a concentration of about 600 µg/kg whereas unflavored rice contains very low amounts (around 10 µg/kg). 2-acetyl-1-pyrroline is identified using the retention indices from AroChemBase database and its quantity is assessed on MXT-5 column on which no visible co-elution is observed. Unflavored rice samples R02 and R03 contain the lowest amounts of 2-acetyl-1-pyrroline (peak area near 0). Among the other samples of flavored rice, the Basmati ones (R01, R06, R07 & R08) show the highest amounts of 2-acetyl-1-pyrroline. The blend of Basmati & unflavored rice (R04) shows a concentration twice lower than pure Basmati rice (R01). The other volatile compounds contained in rice is also investigated using AroChemBase database and it appears that the majority peak corresponds to hexanal, which is an indicator of the level of oxidation.
Polyphenolic compounds are valued for their health benefits and are widely used in the food, nutraceutical and cosmetic industries. These compounds are found in abundance in many natural products and their processed waste materials. Pomace, or marc are the solid remains of grapes, olives, or other fruit after pressing for juice or oil. It contains the skins, pulp, seeds, and stems of the fruit. Grape pomace contains many polyphenolic compounds, such as anthocyanins and procyanidins that have been traditionally extracted using methanol, acetone or other hydroalcoholic solvents.

Liquid water exhibits many changes in its characteristics as it is heated. Viscosity and surface tension decrease and solubility of solutes and their diffusion increases. In addition, the dielectric constant of water decreases significantly as it is heated and behaves like a mixture of water and alcohols when in the subcritical temperature range. Thus subcritical water may be used to extract many organic molecules with substantial environmental benefits compared to the use of conventional organic solvents.
Electronic cigarettes utilize an atomizer to convert a liquid mixture into a vapor, which looks, feels (throat hit), tastes, and produces the desired attributes of conventional cigarette smoke. Little research has been done on electronic cigarettes and the complete chemical components list for these products is unknown. For this reason it is uncertain as to what toxins, if any, could be in electronic cigarette vapor. In this research, we have developed GC-FID and -MS methods for analyzing electronic cigarette liquid and vapor.

Using neat samples of e-juice (electronic cigarette liquid), we were able to develop methods for analyzing both the major and minor chemical components for different electronic cigarette companies. In addition, an exhaustive literature search indicates we are the first to analyze the vapor via thermal desorption. The methods developed for the vapor were used to show cigarette-to-cigarette variability, as well as puff-to-puff variability. These methods yielded both qualitative and quantitative results for the chemical components in electronic cigarettes.
Proteolysis in cheeses has been characterized by chromatographic and electrophoretic techniques in many varieties of cheese. Manufacturing and ripening conditions of many cheeses are differ and these conditions (e.g., type of coagulant, type of starter culture, ripening temperature, ripening period, salting type or salt concentrations etc) significantly influence the quality of cheese. To understand the combined effects of some parameters on cheese quality characteristics, we aimed to produce a model cheese instead of the production of a defined cheese variety in the experimental study. Because, the results from the model cheese study will be more useful and may be ready for the application of any other cheeses. In this context, the influence of adjunct culture type on proteolysis in a model cheese during ripening were studied by taking into account some factors (e.g., type of culture, ripening time, ripening temperature and salt concentrations). The model cheeses were manufactured using the cultures including Lactobacillus helveticus, L. casei, L. plantarum and L. delbrueckii subsp. bulgaricus. Different salt concentrations (1-5%), ripening temperatures (4-20 C) and ripening times (1-120 days) were used as independent variables in the structure of the study. Response surface methodology was used to evaluate the effects of the above independent variables on the proteolysis of the cheese. The levels of proteolysis in cheese were monitored by determining of urea-PAGE of caseins, RP-HPLC of peptides and free amino acids, and soluble nitrogenous fractions. Results showed that the response surface methodology was useful tool to understand the effects of these variables on cheese proteolysis and optimization of experimental design to the next step of the study.

Keywords: Chromatography, Data Analysis, Electrophoresis, Food Science
Application Code: Food Science
Methodology Code: Liquid Chromatography
Determination of Phenolic Compounds in Honeys and Their Antioxidant Capacities

Chemical characterization has been carried out on 30 chestnut honey samples collected from Turkey. The phenolic compounds in chestnut honeys were determined by high performance liquid chromatography-diode array detection (HPLC-DAD). The antioxidant capacities of honeys were measured by 2,2-azino-di-(3-ethylbenzothiazoline-sulphonic acid) (ABTS) and a new chromium reducing antioxidant capacity (CHROMAC) methods.

Thirty phenolic markers were used for chromatographic comparison of data, but only 19 of the 30 phenolic compounds were detected in chestnut honeys. Nine of these phenolic compounds were characterized to be flavonoids and the rest of the compounds were phenolic acids. The phenolic compounds detected in the chestnut honey samples collected from different regions were quite different. Gallic acid, protocatechuic acid, p-hydroxybenzoic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, ellagic acid, hesperetin, quercitrin, salycilic acid, quercetin, kaempherol, apigenin, pinocembrin, crysin, galangin, genkwanin and trans-ferulic acid were detected in chestnut honey. The chestnut honeys are the important antioxidant source for humans because of higher antioxidant capacities determined by ABTS and CHROMAC methods.

The phenolic compound composition of chestnut honey produced in Turkey was reported in detail for the first time in our knowledge. These data could be applied for honey characterization which has great importance for the honey industry due to its important role in the nutritional value of honey.

References

Keywords: Food Science, HPLC, Liquid Chromatography, Spectrophotometry
Application Code: Food Science
Methodology Code: Liquid Chromatography/Mass Spectrometry
Corbicular bee Pollen is an apicultural product, used in human diet for its nutritional value. Daily ingestion of bee pollen is recommended because it is capable to regulate the function of intestines and has benefits on cardiovascular system, skin and vision. Mineral contents of pollen are variable, due to the factors like differences between the plants species, geographical area, and conditions of drying process. The aim of this work has been the determination of the components of the mineral fraction of samples of pollen corbicular in the Andean high plateau of Boyacá. 17 samples from 4 geographical places were collected and analyzed (Tutaza, Sotaquirá, Paipa and Paz del Río), 2200 to 3100 o.s.v.l; 12-14.2 °C). The ashes were determined gravimetrically after dry digestion in furnace at 550 °C. Subsequently dissolved in nitric and chloric acid mixture. Flame atomic absorption spectroscopy we used in analytical determinations for Ca, Cu, Fe, K, Mn, Na and Zn. Additional measurement were made for total nitrogen and phosphorus. Total ash (g/100) dry pollen were between 2,15 ± 0.30 and 2,51± 0.27; mineral ash components in mg/Kg Ca (1461,7-3129,5); Cu (9,30-17,8); Fe (30,1 - 267,6); K (1769 - 5655); Mg (576,7-1023); Mn (4,92 - 49,1); Na (77,4 -211,5) and Zn (37,2 -143,5), with statistical and significant differences (Pv < 0,05) between samples from the same area. Total nitrogen (1,62-3,87) g/100g and Phosphorus (2225- 9790) mg/Kg. The observed differences between the samples may be explained under the botanical origin and the characteristics of the soil in each geographical area. The work contributes to the study of the products of the hive and the differentiation of Colombian types of bee pollen, a natural product of wide demand for human consumption.

Keywords: Atomic Absorption, Atomic Spectroscopy, Food Identification, Food Science
Application Code: Food Science
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Transmittance Spectra Color and Chromatic Properties of Edible Oils and Changes Induced by Thermal Treatment

Sensory attributes of color and physicochemical properties of refine vegetable oils are important contributing factors in the determination of their market value. Lovinond system has been used as a tool in the industry of the edible oils for final quality control of color. Many difficulties in the use of this system have arising in practice; in that case the aim of this research was focused in the evaluation of transmittance spectra of commercial edible oils in Colombia. Additional characteristics were defined for their chromatic properties of luminance (L), red/green (a*), yellow/blue (b*) and chroma that were measured in Corn, Olive, Rapeseed, Soybean and Sunflower refined and oils under thermal treatment between 210-220ºC. Complete information on the chromatic and color properties of a particular oil derived from the transmittance spectra between 400 and 700 nm, with specific shapes according quantity of pigment ported by the seeds. The well-defined absorption band centered for the oils at 450 and 550 nm. The luminance parameter was low in Olive and extra virgin oil. On the other hand chromaticity (a*; b*) for Corn (-1.26; 23.3), Olive (-7.48; 23,3), Rapeseed (-4.92; 9.74), Soybean (-5.87; 9.51) and Sunflower (-3.03; 5.92) showed significant differences. Chroma properties were (24.5) Olive and Corn (23.3), Rapeseed (10.9). The order for luminance was (89.9) < Corn (98.2) < Soybean (99.1) < Olive (98.2). Chromatic properties and shape of transmittance spectra in every each oil change under thermal treatment. Physicochemical properties in fresh oils change after their use in frying process.

Abstract Text

Data Analysis, Food Science, UV-VIS Absorbance/Luminescence

Application Code: Food Science

Methodology Code: UV/VIS
The flavor and odor profile of scotch whiskeys is influenced heavily by the presence and relative concentration of sulfur-containing compounds contained therein, which include dimethyl sulfide, dimethyl disulfide, 3-(methylthio)propanol, benzothiophene, and many others. The aging process affects the concentrations of numerous species, and we will present both the key sample introduction details of our analysis, as well as an aging time-dependent concentration map of some of the principle flavor component sulfur compounds present in a selected set of aged and un-aged whiskeys. Performance data collected using a next-generation pulsed-flame photometric detector (PFPD) will be compared with data from a commercially-available PFPD, which has previously demonstrated good utility for sulfur analysis in other applications.

**Abstract Text**

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**Keywords:** Flavor/Essential Oil, GC Detectors, Sample Preparation, Sulfur

**Application Code:** Food Science

**Methodology Code:** Gas Chromatography
Pectin is a high value functional food ingredient widely used as a gelling agent and stabilizer in foods. The aim of this study focus on the microstructure changes in orange and mango peels after essential oil and pectin extraction using conventional heating and microwave (mw). Structural changes of the peels were related with the extraction yield, the degree of esterification and the percentage of free carboxyl groups. Mango and orange fruits were used in this research. Scanning electron micrograph for orange and mango peel before and after of treatment was evaluated. Epicarp of fruits were stored at -20 °C, and cut into sizes of 0.5 x 2 cm. The essential oil extraction was performed in a glass vessel of 2.5 L., coupled to a clevenger device which was cooled at -5 °C in a microwave of 2.45 GHz at 600 W for 10 and 20 min. In pectin extraction with conventional heating, epicarp, was treated with acidulated water (10:1), then the enzyme inactivation was carried put at 80 °C for 10 min. and the extraction conditions were at pH, 2.0 and 80°C, by 55 min, (mango) and pH, 1.5, 85°C, 55 min, (orange). Microwave heating extraction, were performed at the same conditions, but applied 500 W and 600 W for mango and orange. Pectin was precipitated with 96% alcohol (1:1), filtered, and washed with ethanol (70 and 96%); finally, the solid was lyophilized. Scanning electron micrograph for orange and mango peel before and after of treatment was made at SEM JEOL, Model JSM 6490LV. Electron source with tungsten filament (0.3 - 30 kV). Secondary electron detector, a backscattered electron detector and an X-ray detector for ESD. The sample of 1 cm x 1 cm surface area was coated with gold in a thickness of 120-150 austrong during 75 s, using sputtering technique in coating equipment.

Keywords: Analysis, Chemical, Food Science, Nanotechnology
Application Code: Food Science
Methodology Code: Separation Sciences
Food Sciences

Determination of Hexanal in Foods Utilizing Dynamic Headspace GC/MS

Hexanal is one of many well-documented aromatic components that contribute to flavor and aroma in common consumer food products containing omega-6 fatty acids. Hexanal content is also used to measure the oxidative status of foods rich in omega-6 fatty acids.

Dynamic headspace sampling differs from static headspace sampling in that the headspace above the sample is swept with a gas onto an absorbent trap, rather than allowing a sample and its headspace to reach a static equilibrium before transferring the volatile compounds to a gas chromatograph/mass spectrometer (GC/MS). Employing dynamic headspace sampling provides all the benefits of static headspace with the low-level detection capabilities similar to analysis by purge and trap.

The hexanal content of two common consumer food products, rice and infant formula, was evaluated for hexanal content. Dynamic headspace was used to concentrate hexanal from food products, minimizing labor intensive solvent extraction and clean up. The hexanal was then separated from other volatile compounds and quantitated with a GC/MS.

Keywords: Food Safety, Gas Chromatography/Mass Spectrometry, Headspace, Purge and Trap
Application Code: Food Science
Methodology Code: Gas Chromatography/Mass Spectrometry
The analysis of wine aromas is a complex process. A reference to a wine’s aroma is indicative of the scents associated with the grapes used to make the wine, while the bouquet is an indication of the aging process of the wine. There are hundreds of compounds that can contribute to the overall aroma of wine. In order to determine the aroma compounds, analysts can use static headspace, dynamic headspace, Solid Phase Micro Extraction (SPME), liquid-liquid extraction, etc... This analysis will compare SPME fibers and the efficiency of the SPME fiber coating for wine aroma analysis.

Keywords: Food Science, Gas Chromatography/Mass Spectrometry, Sampling, SPME
Application Code: Food Science
Methodology Code: Sampling and Sample Preparation
There are a large variety of volatile compounds that can be found in alcohol. These volatile compounds can add or deter from the flavor of your drink. Trained analysts can often detect the flavors in liquors by smelling and tasting a sample. However, in order to establish the quality and essence of a liquor sample, further testing is required. Gas Chromatography coupled with Mass Spectrometry (GC/MS) is an effective tool for the determination of the flavor compounds in an alcohol. Utilizing different sampling techniques, the respective efficiency in the determination of flavor compounds in whiskey samples will be investigated.

**Keywords:** Flavor/Essential Oil, Food Science, Headspace, SPME

**Application Code:** Food Science

**Methodology Code:** Sampling and Sample Preparation
With the introduction of SPME in 1990, Food/Flavor/Aroma/Fragrance Chemists were able to see more compounds than ever before, as strong reactive adsorbents could be avoided. Unfortunately, there are several limitations to this technique, brought about by competitive absorption by the matrix, slow uptake of heavier volatiles, fiber swelling and damage by high alcoholic matrices, excessive carryover, and poor sample throughput when performing high sensitivity SPME analyses.

An improved version of SPME, called “Porous Cartridge Micro Extraction (PCME)” is presented here that eliminates many of the shortcomings of SPME. PCME uses a ceramic coated porous metal cartridge containing far more surface area than a SPME fiber. The cartridge can be coated with absorbents such as PDMS, and has a 1/8” hollow interior that holds up to 100mg of Tenax TA or other adsorbents. PCME has a flow through design that allows creation of a vacuum after the cartridge is inserted into the sample headspace to vastly increase the diffusion rate from the liquid/headspace boundary to the cartridge. Vacuum extractions can occur 4-8 times faster, especially for heavier aromas or off-odors such as Geosmin and 2-methyl-isoborneal (lake water smell) in drinking water with detection down to as low as 1-2 part-per-trillion. Several PCME cartridges can sample simultaneously for improved sensitivity when performing low part-per-trillion level headspace analysis. Data on Geosmin and 2-methyl-isoborneal will be presented, showing a vast improvement in sensitivity and sample throughput relative to classical SPME analysis.

Keywords: Flavor/Essential Oil, Gas Chromatography/Mass Spectrometry, Headspace, SPME
Application Code: Food Science
Methodology Code: Sampling and Sample Preparation
Study of Magnetic-Bead Systems to Remove Peanut Allergens

Peanut allergens are known to bind to phenolic compounds, hydrophobic surfaces, and molecules with ionic charge such as ferric ion (Fe3+). In this study, we prepared magnetic-bead systems with each of these binding properties in hopes that they could remove peanut allergens from a peanut extract. Magnetic beads covalently linked with the phenolic compound, gallic acid (GA), were prepared by incubating aminoethyl beads with GA in the presence of carbodiimide. Hydrophobic beads were purchased commercially. The GA beads and hydrophobic beads were incubated with a peanut extract. For the ferric iron treatment, plain magnetic beads were incubated with a peanut extract that had previously been incubated with ferric sulfate and dialyzed. After incubations the beads were retrieved by using a magnetic device to separate beads from the extracts. Proteins bound to the beads were eluted using 1 M NaCl. Retrieved extracts and eluted proteins were analyzed by SDS-PAGE and ELISA, using a pooled plasma from peanut-allergic individuals and a microplate reader. Results showed that much of the two major peanut allergens were removed by the GA system, while some of these allergens were removed by the Fe3+ and hydrophobic systems. Overall, the allergenic capacity (i.e., IgE binding) of the treated extracts were reduced by 30-56% and GA appeared to be the best. In conclusion, the extent of allergen removal depended on the ligands used. The magnetic beads provide a simple way to remove peanut allergens, and could serve as a model system for the development of hypoallergenic products.

Keywords: Food Safety, Food Science, Method Development, Protein
Extracts from natural product samples can be complex often containing a large number of diverse compounds. Increased separation performance of sub-2-μm column technology along with low dispersion instrumentation provides a tool that produces sharp, narrow, and more concentrated peaks. When there is a need to collect narrow peaks from these complex mixtures, traditional fraction collection instrumentation designed for preparative HPLC conditions does not provide an adequate solution.

Extracts from several natural product samples were analyzed using sub-2-μm chromatography. Potential peaks of interest were identified and isolated using a fraction collector designed to overcome the limits of traditional fraction collectors. Collection of narrow peaks generated using modern sub-2-μm chromatography along with closely eluting compounds is demonstrated using a variety of collection modes including time based and peak detection techniques. Analysis of collected fractions to demonstrate purity is shown using both LC and SFC methodologies.

Keywords: Chromatography, HPLC, Natural Products
Application Code: General Interest
Methodology Code: Liquid Chromatography
Abstract Text

Molecularly imprinted polymer (MIP) is very useful in separation and sensing fields. As the MIP is usually prepared by the polymerization of the mixture of template, functional monomer, and cross-linking agent, the rigid polymer should be ground to increase the uptake of the target molecule. This process is very tedious and time consuming. We prepared MIP in the fine powder state by the complexation between polymer anion and metal cation using the process of solvent-nonsolvent treatment. The obtained fine powder has higher uptake of template up to the theoretical value. The polymer was prepared with methymethacrylate (MMA) and acrylic acid (AA), and dissolved in dioxane. Template was added into the solution to bind to the polymer, then added metal cation to form a complex. By the addition of non-solvent, the fine powder of MIP was obtained. This process is relatively easier to increase the uptake.

Keywords: Adsorption, Chiral Separations, Environmental Analysis, HPLC Columns

Application Code: Environmental

Methodology Code: Separation Sciences
Molecularly imprinted materials (MIP) is very useful in the separation process but has the problem how to maximize uptake of the template. To increase the uptake, MIP should be obtained in the fine powder state. In this work, MIP was prepared in the form of star-like polymer on the silica surface. The fine particles of TEOS/3-(methoxysilyl)propyl methacrylate which was obtained by sol-gel method was polymerized together with the mixture of MMA/AA in dioxane. In the solution, there are two types: polymer part of MMA/AA and grafted polymer part on silica surface. After removing the polymer of MMA/AA, the fine particles of sol-gel/polymer was obtained. To the sol-gel/polymer, template was imprinted in THF solvent. Then by the addition of metal ion, sol-gel/polymer MIP was prepared. The uptake was very higher close to the theoretical value.

Keywords: Adsorption, Chiral Separations, Environmental Analysis, HPLC Columns
Application Code: Environmental
Methodology Code: Separation Sciences
Abstract

Hydrazine compounds are known to exhibit toxicity and can potentially damage DNA with low levels of exposure. Hydrazine and alkylated hydrazines are not easily quantified using traditional liquid chromatography as they lack a chromophore to enable traditional UV detection, and gas chromatographic analysis can be troublesome as well due to the high volatility and reactivity of hydrazines. Derivatization is often employed to enable the quantitative analysis of hydrazine and alkylated hydrazines. The derivatizations undertaken in this study had to be easy, fast, and sensitive. Current work has focused on developing and employing facile derivatization to permit quantitative analysis of hydrazine using traditional chromatographies at parts per billion levels from solid matrices.

Keywords: Derivatization, GC, Liquid Chromatography, Trace Analysis
Application Code: Other
Methodology Code: Liquid Chromatography
The improvement in efficiency observed with solid core silica-based substrates comes from the greater uniformity in the particle substrate, which allows for better packing efficiencies, a reduction in the void volume of the column which lowers the longitudinal diffusion and also the reduction in mass transfer effects due to the reduced pore depth offered by the solid-core material. This poster will examine the contributions of all of the three dispersion terms and determine if the current models match the practical gains that have been previously demonstrated.

An investigation on the effects of particle size will also be performed, focussing on three sizes ranging from 4 \( \text{m} \) down to sub 2 \( \text{m} \). This will look at how scalable the efficiency gains are in comparison with fully porous materials and also investigate the effects of extra column dead volume. Example separations will be used to investigate the effects that reducing the particle size can have on the selectivity. It has been previously reported that increases in the pressure within a chromatographic system can affect the retention mechanism due to a shift in the equilibrium of the analyte stationary phase complex formation process, and this will be investigated with a range of different compounds to see if this is a phenomena that separation scientists have to consider in the transfer of methods. The use of smaller particles creates higher pressure drops across the columns which will result in increased temperature gradients. This will be investigated to determine the effect that the pressure induced temperature gradients can have on the performance of the separation, and the effects that running the system in a nominally isothermal mode compared to performing the same separation in an adiabatic mode will have on the overall performance of the separation. To establish these two different modes of temperature control a new column oven which allows for forced air heating and still air heating will be employed.

Keywords: HPLC, HPLC Columns, Liquid Chromatography
Application Code: General Interest
Methodology Code: Liquid Chromatography
Can Core-Shell Silica Boost Efficiency of Macrocyclic Oligosaccharide Based HILIC Phases?

Core-shell silica has been raising the bar for high efficiency separations in reversed phase for several years now. Currently, other modes of chromatography are attempting to take advantage of core-shell silica as well. To date, almost all core-shell silica based “HILIC” columns are simply unmodified silica. Further, the impact of the mobile phases applied in HILIC on the advantages of core-shell silica have not been well studied. In this work, HILIC stationary phases comprised of specifically designed cyclic oligosaccharides bonded to superficially porous silica particles were developed. Their performance in HILIC was evaluated and compared to fully porous silica particles with 5um and 3um diameters. Faster and more efficient chromatography was achieved. The columns were also evaluated in the normal phase mode. The peak efficiency, analysis time, resolution, and overall separation capabilities in both HILIC and normal phase modes are compared. At higher flow rates, the mass transfer advantage of the superficially porous particles was more pronounced in normal phase separations than in HILIC, demonstrating clearly the influence of the mobile phase in the mass transfer of the analyte. Yet, the minimum reduced plate heights were typically lower in HILIC than NP. Overall, the superficially porous particle based columns showed advantages over the fully porous particle columns, in terms of high throughput and efficient separations at high flow rates, allowing for very fast separations to be performed.

Keywords: HPLC, HPLC Columns, Liquid Chromatography, Modified Silica
Application Code: General Interest
Methodology Code: Liquid Chromatography
Fluorinated stationary phases are commercially available and are useful in separations of compounds of interest to the pharmaceutical as well as other industries. Although a number of literature studies have appeared, the retention characteristics of these phases are not well characterized, and few comparisons between fluorinated and similar non-fluorinated phases have been reported. Retention of approximately 40 aromatic analytes with phenyl, alcohol, nitro, cyano, halogenated, and other functionality was measured using a fluorophenyl stationary phase at temperatures ranging from 5.0 to 65.0°C and with a mobile phase composition of 10% methanol/90% water (v/v). By analyzing the retention data through van’t Hoff and selectivity plots, the effect of temperature and the thermodynamics of the retention process are presented. To demonstrate how this stationary phase is affected by mobile phase composition and how the retention behavior may be different than for other similar phases, similar results will be presented for other mobile phase conditions, such as by changing the organic component to acetonitrile or varying the organic/water ratio, and to using a phenyl stationary phase under the same conditions.
The feature of superficially porous particle used as a highly efficient material is existence of a core, a thin porous layer and narrow particle size distribution, which lead to higher efficiency than totally porous particle. On the other hand, recently a monodisperse silica gel has been presented to be the almost same particle size distribution as a superficially porous silica and to be higher performance materials than a conventional totally porous silica. In this study, a monodisperse totally porous silica and a superficially porous silica were compared regarding theoretical plate and Van Deemter plot. As a result of a plate measurement, a monodisperse silica showed 16% higher theoretical plate than a conventional totally porous silica, while a superficially porous silica indicated 50% higher. It was leaded by comparing with Van Deemter plot that only A term of Van Deemter Equation was decreased by effect of narrow particle size distribution and both B and C terms were reduced by effect of a thickness of porous silica layer. It was elucidated that predominance of superficially porous silica over totally porous silica was leaded by not only low Eddy diffusion due to narrow particle size distribution but also both low longitudinal diffusion and short mass transfer path due to a thin porous layer.

Keywords: HPLC Columns
Application Code: General Interest
Methodology Code: Liquid Chromatography
Recent spectrometric studies have shown that stable nanobubbles, bubbles of submicrometer size, can easily be trapped inside hydrophobic cavities of alkyl-bonded silica. This phenomenon can be explained in terms of capillary effect and the cavity geometry.

It has been reported that aqueous solutions are frequently expelled from the pores of hydrophobic porous materials in reversed-phase liquid chromatography (RPLC). This result is consistent with the observation of spectrometric studies and the pores from which liquid water has been excluded may be occupied by a gas phase.

The separation mechanism in reversed-phase systems has been explained by various models e.g. adsorption of analytes onto the hydrophobic surface and partition into the hydrophobic moieties. On the other hand, Shibukawa et al. showed that separation of inorganic ions and hydrophilic organic compounds in the reversed-phase systems can be successfully explained by the partition between the hydrophobic interfacial water and the bulk water [1]. The nanobubbles fixed in the pores may also act as a separation medium for volatile compounds. This means that we are able to make a hybrid separation medium consisting of the interfacial water, hydrophobic moieties and nanobubbles.

In this paper, we will present a new liquid chromatography with the hybrid stationary phase consisting of the bubbles, the interfacial water and the hydrophobic moiety, surface bubble modulated liquid chromatography (SBMLC). The retention volumes of various compounds and the volume of the interfacial water depend on the back pressure of the column. This indicates that, in aqueous mobile phase systems, the interface between the bonded layer and water or the hydrophobic interfacial water has a key role in retention and that the retention selectivity can be easily manipulated by the applied pressure on the SBMLC column.


Keywords: HPLC, Liquid Chromatography, Water
Application Code: General Interest
Methodology Code: Liquid Chromatography
A novel two-dimensional LC (2D LC) has been developed and successfully applied to phospholipids, polyphenols and medicinal compounds in biological, food and crude drug samples respectively. A comprehensive 2D LC is recognized as very effective strategy for both quantitative and qualitative analyses, especially for natural and biological samples in complex matrices. However, the combined effect of the necessity to minimize band broadening in the 1st dimension and the very short analysis time in the 2nd dimension has been a difficulty for spreading this technology. Recent progress in the technology of solvent delivery at very low flow rate and ultra-high speed HPLC (UHPLC) analysis realizes a practical and comprehensive 2D LC. Our new, truly comprehensive 2D LC system, “Nexera-e”, combines our latest UHPLC Nexera X2 product line with photo diode array and/or mass spectrometric detectors. Comprehensive 2D LC allows for highly efficient separation of a variety of samples, even in complex matrices, as well as ordinary quantitative analysis. The orthogonal separation selectivity between 1st and 2nd dimensions is a key issue for this technique but difficult to accomplish due to solvent compatibility and column selection in the 1st and 2nd dimensions. In our study for improving the limited orthogonality, it showed dramatic improvement when employing a shifted gradient profile in the 2nd dimension, where initial and final mobile phase composition increase stepwisely, to match the 1st dimension gradient profile. Raw data is mathematically manipulated by dedicated software, resulting in enhanced peak capacity and identification ability compared to those of independent 1D and 2D separations.

Keywords: Food Science, HPLC, Lipids, Pharmaceutical
Application Code: General Interest
Methodology Code: Liquid Chromatography
Comparison of the Retention Behavior of Fluorinated and Traditional, Hydrocarbon HPLC Stationary Phases Using Linear Solvation Energy Relationships (LSER), Cluster Analysis, and k–k Plots

Fluorinated HPLC stationary phases are commercially available and useful in separations of fluorinated compounds and compounds of biological interest. Although the literature contains a number of studies using fluorinated stationary phases, a complete understanding of how fluorinated stationary phases interact with analytes has not been obtained. Retention of over 40 aromatic analytes with phenyl, alcohol, nitro, cyano, halogenated, and other functionality have been measured on fluoro octyl, octyl, phenyl, and fluorophenyl stationary phases. The measurements were made at temperatures ranging from 5.0 to 65.0 °C and with various compositions of organic (methanol or acetonitrile) and water mobile phase mixtures. Differences in retention and how retention changes in response to temperature and mobile phase composition have been observed when using fluorinated and similar hydrocarbon phases. To gain a better understanding of these differences, a number of multivariate analysis techniques, such as cluster analysis and principal component analysis; k–k plots; and linear solvation energy relationships (LSER) have been applied to this data. The cluster analysis and k–k plots emphasize similarities and differences in the retention behavior without providing much direct evidence of the underlying processes that lead to retention while LSER provides more information on the importance of the various interactions that lead to retention. We will discuss the advantages and disadvantages of using these various analysis techniques, show some of the similarities and differences in the retention behavior when using these four stationary phases, and start providing a potential explanation of the interactions that lead to the observed retention behavior.

Keywords: Chromatography, HPLC, HPLC Columns, Liquid Chromatography
Application Code: General Interest
Methodology Code: Liquid Chromatography
A column packed with core shell particles has been widely used on HPLC and UHPLC, because it showed not only excellent column efficiency but also lower back pressure than sub-2 um column. More than 20 kinds of core shell column are available in the market. It is said that two types of core shell silica particle are used. One is a mono-layer structure as a porous silica layer like a core shell silica manufactured by Advanced Materials Technology and another is a multi-layer structure like that manufactured by Phenomenex using so-called layer-by-layer method.

In this study, a separation behavior, stability and physical property for Kinetex C18, Accucore C18, Cortecs C18, PoroShell C18 EC, Ascentis Express C18 and SunShell C18 were evaluated. Retention factor, hydrogen bonding capacity, hydrophobicity and steric selectivity were measured using Tanaka method. Not only peak shape of neutral, acidic and basic compounds but also loading capacity of amitriptyline under neutral and acidic conditions were also measured. Furthermore stability under acidic pH1 and basic pH10 conditions was evaluated. Regarding physical property, carbon loading of each C18 packing material, and specific surface area, pore volume, pore diameter of each core shell silica which was deleted alkyl chains by calcination at 600 degree Celsius for 8 hours were measured. As a result, the big difference was recognized among 6 kinds of core shell C18s for separation behavior, stability and physical property. This difference is considered to be due to each manufacturing method and bonding technique as well as fully porous silica C18s. SunShell C18 showed the largest retention factor and the highest stability though its carbon loading is not the highest, while Kinetex C18 showed the lowest retention factor, the lowest carbon loading and the lowest specific surface area.
Thermal Analysis

Investigating Unknown Polymer Mixtures By DSC Using New “Identify” Software

Software for identification of unknown materials by comparison against known-sample databases or spectral libraries has been standard for FTIR, MS, and other techniques almost since the introduction of the personal computer. For Differential Scanning Calorimetry (DSC) this has not been the case, perhaps due to the seemingly limitless variables which can affect scan results such as heating rate, purge gas type and flow rate, crucible type, etc. Recently, however, a powerful new DSC curve recognition and interpretation software called “Identify” has been introduced which allows for DSC curve comparison against a built-in database system providing polymer identification with a single click. This is useful for formulation studies and also for quality control and quality assurance. This poster will detail how the new “Identify” software is applied to DSC results to identify the composition of polymeric materials and mixtures.

Keywords: DSC, Identification, Materials Characterization, Thermal Analysis
Application Code: Polymers and Plastics
Methodology Code: Thermal Analysis
The polyethylenes (LDPE, LLDPE, HDPE) are the most industrially produced synthetic polymers in volume with a production of around 75 MT a year. The LLDPE which is produced by coordination catalysis is a copolymer of ethylene with a alpha-olefin (typically hexene or octene). One of its main advantage is that its structure and consequently its properties are easily adjustable by variation of the co-monomer content (short chain branching). It is thus of high interest to determine the structure of LLDPE. A range of crystalline copolymers of ethylene with alpha-olefins (propene, hexene, octene, octadecene and norbornene) were prepared using the complexes Et(Ind)2ZrCl2 and (nBuCp)2ZrCl2 activated with MAO. The average composition of the copolymers was measured using 1H, 13C NMR and TREF. The samples were characterized by coupling of the TGA with the GC-MS technique to get information about the structure of LLDPE.

For a clearer understanding of certain reactions, the TGA technique is often coupled to mass spectrometry (MS) and infrared spectroscopy (IR) for identifying the gaseous compounds emitted during thermal decomposition of the sample. When it comes to complex materials such as LLDPE, then gas mixtures are produced but the majority of components cannot be identified precisely with MS or IR. In this case, coupling of the TGA with the GC-MS technique offers interesting advantages. The emitted compounds are first separated by gas chromatography (GC) then identified and quantified by MS. An innovative TGA/IST16/GC/MS coupling is presented that significantly increase the number of data collected and thus provides an efficient way to take advantage of the GC/MS technique. The configuration uses a fractions collector inserted between the TGA and the GC. This coupling is a powerful and versatile tool for interpretation of structure of LLDPE. An example with characterization of several polyethylene samples will illustrate this.

Keywords: Characterization, Gas Chromatography/Mass Spectrometry, Pyrolysis, Sample Introduction
Application Code: Polymers and Plastics
Methodology Code: Thermal Analysis
Although the use of chemical warfare nerve agents (CWNA), such as sarin, and VX is forbidden by the Chemical Weapons Convention, documented cases of the use of these nerve agents exist. For infants and small children, the risk for inhalation and dermal exposure to CWNA may be greater than adults due to greater minute ventilation rates and larger surface area to body mass ratios, respectively. The developing nervous system is particularly vulnerable to xenobiotics that disrupt the cholinergic system. Numerous studies have been done on the developmental neurotoxicity in rats of organophosphate pesticides, such as chlorpyrifos. However, there have been relatively few studies on the developmental neurotoxicity of CWNA exposure. In support of efforts to characterize the behavioral and neuropathological effects associated with developmental exposure to sarin, the biomonitoring of sarin in various biological matrices from rats following whole-body inhalation exposure has been evaluated using the technique of fluoride ion regeneration followed by GC-MS/MS analysis.

Extracts of indicative biomarkers were obtained from blood and tissues from rats exposed to sarin via an inhalation route (60 minutes at 0.6xLC50) using fluoride ion to release bound agent from adduct sites, isolating the agent with a C18 solid phase extraction column, and eluting with ethyl acetate. Prior to extraction, tissue samples were subjected to freeze-fracture pulverization under cryogenic temperatures and homogenized using focused acoustic energy. The extracts were analyzed by chemical ionization gas chromatography tandem mass spectrometry. The multiple reaction monitoring mode was used for detection, and quantitation was accomplished with a deuterated stable isotope as an internal standard.

Results presented will show the successful application of the method to the analysis of blood and tissues from postnatal day (PND) 14, PND 21, and PND 42 rats taken at selected time points following exposure.

Keywords: Biological Samples, GC-MS, Toxicology
Application Code: Clinical/Toxicology
Methodology Code: Gas Chromatography/Mass Spectrometry
Melamine, a nitrogen-rich chemical, (2,4,6-triamino-1,3,5-triazine) was intentionally added by some company to foods (liquid milk or milk powder) and animal feed to boost the apparent protein content in 2007. Today, pet foods and infant formula products, contaminated with melamine, have created a widespread food safety scare1,2.

The detection of melamine with MIP has been the subject of intense research. In this study, melamine imprinted polymeric films were prepared on Quartz Crystal Microbalance (QCM) chip. Firstly, template molecule (melamine) and functional monomers (acrylamide and methacrylic acid) were preorganized and then, preorganized complex and crosslinker monomer (Methylenebisacrylamide) were polymerized with UV irradiation. The MIP nanofilms were characterized by SEM and atomic force microscopy. After the characterization studies, the imprinted and non-imprinted QCM sensors were connected to QCM system to obtain the kinetic and affinity studies for the target molecule. Imprinted nanofilms showed more sensitivity to melamine than non-imprinted ones. Langmuir adsorption model was found as the most suitable model for this affinity system. In order to show the selectivity of the melamine imprinted nanofilms, competitive adsorption of melamine and cyromazine was investigated. The results showed that the imprinted nanosensor has high selectivity and sensitivity for melamine.

Acknowledgements
This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK): grant number KBAG-112 T 561.

Identification of drugs of abuse is an increasing challenge for analytical laboratories. Liquid or gas chromatography mass spectrometry (MS) is often utilized for this analysis. Many drugs are structural isomers and cannot be distinguished by MS of the intact ion. Chromatography can often resolve isomers at the cost of increased analysis time. Collisionally induced dissociation (CID) in the mass spectrometer may distinguish structural isomers without use of chromatography. This study was performed to determine if direct sample analysis (DSA) combined with high mass accuracy TOF MS analysis of CID fragments can differentiate isobaric drugs in mixtures. Sample analysis was performed using a PerkinElmer AxION 2 TOF MS fitted with a DSA source. Collisionally induced fragmentation was induced at the capillary exit of the TOF. Analytes were prepared in methanol and applied to a stainless steel mesh for DSA-TOF analysis using AxION DSA Controller AxION Solo software. 12 isobaric drug pairs were analyzed in this study. DSA-TOF analysis distinguished 11 of the isobaric pairs using unique fragment ions. For example, scopolamine and cocaine (C17H21NO4) were uniquely identified using fragment ions at m/z 138.0918 (scopolamine) and m/z 182.1185 (cocaine). In addition, scopolamine and cocaine were mixed in variable proportions and the diagnostic ions varied with analyte concentrations. One isobaric drug pair, O-Desmethylvenlafaxine and Tramadol, did not produce unique fragment ions but could be distinguished using differences in the relative abundance of a shared fragment ion. These results demonstrate that isobars can be rapidly distinguished without chromatography using DSA-TOF analysis of CID fragments.
Toxicology

A Reduced Workflow Solution for the Analysis of GHB in Human Hair Samples via an Automated Bead Mill as a Precursor to High Resolution GC-TOF and GCxGC-TOF

Techniques to interrogate hair samples have proven valuable in detecting human host exposure to drugs of abuse over a long sampling window. The hair matrix is challenging to work with and a number of methods have been reported. This investigation details the feasibility of incorporating an automated bead mill to homogenize the matrix and disrupt non-selective analyte binding on a molecular level by destroying the matrix on a macro-level. It was determined that the bead mill was effective in improving the efficiency of the subsequent digestion familiar to hair testing protocols. Proof of concept was determined using adult human head hair samples (n=5) which were prepared in duplicate using the laboratories standard preparation procedure (cutting the hair into 1-2mm segments using scissors) versus automation in the bead mill at the Center for Forensic Science Research and Education (Willowgrove, PA) using a Biotage BeadRuptor 24. The homogenized samples were incubated overnight in methanol at 40 ºC, filtered and evaporated to dryness. The sample tubes were sent to Leco’s application lab (Saint Joseph, Michigan) for derivatization and various GC-TOF platform analyses) followed by post acquisition deconvolution software. A GCxGC approach was employed to mitigate a co-eluting peak tentatively identified by PBM software as TMS-urea. Preliminary data suggests alternatives to GCxGC may be found in SPE techniques prior to single stage GC-MS. Additional samples from a range of individuals (age and sex) were similarly prepared and tested via the optimized GCxGC-TOF method showing the method to be effective in separating GHB-2TMS from endogenous interferences. This presentation will have impact on the forensic community by increasing awareness of alternative instrumental techniques that can be used to optimize the determination of silylated-GHB in hair.

Keywords: Automation, Clinical/Toxicology, GC-MS, Sample Preparation

Application Code: Clinical/Toxicology

Methodology Code: Sampling and Sample Preparation
There are several ways to determine the amount of alcohol that is in a person’s system. The most common methods are breath analysis in the field and blood analysis in the lab. Blood alcohol determination in the laboratory is used predominantly when a person refuses a breath test. In order to determine blood alcohol content, a person’s blood has to be withdrawn as soon as possible after the occurrence. Furthermore, the blood needs to be collected in duplicate in order to confirm the test results. This application note will examine static headspace sampling of alcohol standards using Gas Chromatography (GC) for separation and Flame Ionization Detection (FID) for analysis. The linearity of the compounds of interest will be examined and compared using a secondary column for confirmation. Additionally, as many forensic labs have an excess of samples to examine, the use of software innovations will aid in optimizing sample throughput.

**Abstract Text**

There are several ways to determine the amount of alcohol that is in a person’s system. The most common methods are breath analysis in the field and blood analysis in the lab. Blood alcohol determination in the laboratory is used predominantly when a person refuses a breath test. In order to determine blood alcohol content, a person’s blood has to be withdrawn as soon as possible after the occurrence. Furthermore, the blood needs to be collected in duplicate in order to confirm the test results. This application note will examine static headspace sampling of alcohol standards using Gas Chromatography (GC) for separation and Flame Ionization Detection (FID) for analysis. The linearity of the compounds of interest will be examined and compared using a secondary column for confirmation. Additionally, as many forensic labs have an excess of samples to examine, the use of software innovations will aid in optimizing sample throughput.

**Keywords:** Forensic Chemistry, Gas Chromatography, Headspace, High Throughput Chemical Analysis

**Application Code:** Clinical/Toxicology

**Methodology Code:** Gas Chromatography
Glucose is the essential nutrient in vivo metabolism because the heat released by its oxidation is an important source of energy for human life. Blood glucose level is also an indicator of human health conditions, and it should maintain a certain concentration in order to maintain the body organs and tissues need [1].

Colorimetric biosensing has attracted much attention due to its low cost, simplicity, and practicality. Since color changes can be discriminated by naked eyes, colorimetric biosensing does not require expensive or sophisticated instrumentation and can be applied to field analysis and point-of-care diagnosis [2,3].

In this study, 2-aminothiazole was polymerized via enzymatic polymerization with bi-enzyme system in aqueous solution containing iron nanoparticles (FeNPs). Poly (2-aminothiazole) covered bi-enzyme system was characterized and used for glucose determination by spectroscopic method. Some parameters for glucose determination were also optimized.

References:

Keywords: Biosensors, Polymers & Plastics, Spectrophotometry
Application Code: Bioanalytical
Methodology Code: UV/VIS
Globular proteins are transformed from the native state into the intermediate states or into the denaturated state under different conditions as low pH, high concentrations of denaturant, heating, etc. Despite some common features, a coherent picture of the intermediate state is still subject of debate and independent investigations are necessary for each case. In this study, pH-induced transition of holo-form of bovine \( \alpha \)-lactalbumin from the native to the intermediate state was analyzed by probing its tertiary and secondary structure using concerted spectroscopic approach. 2D correlation spectroscopy combined with principle component analysis (PCA) can provide deeper insight into the changes developed at level of tertiary and secondary structure of protein. To better understand transition native to intermediate state of \( \alpha \)-lactalbumin, 2D correlation analysis was applied to the pH-dependent IR and Raman spectra of \( \alpha \)-lactalbumin. In this presentation, details of pH-induced transition of \( \alpha \)-lactalbumin will be discussed.
The effects of time, temperature, and humidity on the stability of pain-relieving components in children's nonsteroidal anti-inflammatory drugs (NSAIDs) are the foci of this presentation. To minimize the decomposition of pain-relieving components in NSAIDs, selection of appropriate storage conditions to prolong the effectiveness of the painkillers is essential to the health of infants and young children. Acetylsalicylic acid (aspirin), the most commonly used analgesic, is available in prescription and over-the-counter forms. The degradation of aspirin into salicylic acid and acetic acid renders the aspirin both ineffective for treating pain and harmful to consumers. The goals of this research are: (1) to assess the effects of time, temperature, and humidity on pain-relieving components in children’s NSAIDs, and (2) to supply parents and health care providers with information on safe storage conditions for painkillers.

The NSAIDs to be studied will be subjected to storage conditions which span various levels of time, temperature, and humidity. The quality of aspirin in the NSAIDs, in terms of acetylsalicylic, salicylic, and acetic acid concentrations, will be determined by spectrometric (e.g. second-derivative and multicomponent spectrophotometry) and chemometric (e.g. principal component analysis (PCA) and regression techniques such as ordinary least squares (OLSR), partial least-squares (PLSR), and principal component (PCR) regression) methods. Sample preparation, multivariate calibration, and analytical determination methodologies will be presented and discussed, as will the results of the study to date and their significance.

Keywords: Chemometrics, Drugs, Pharmaceutical, Spectrophotometry
Application Code: Pharmaceutical
Methodology Code: UV/VIS
Acid rain hurts growth of plants, because it damages not only cells/tissues of plants but also the metabolism such as the activity of the enzyme. Here, we used the optical beam deflection method and absorption spectrometry for investigating effects of acid solution on a plant. Egeria was used as a model plant. In absorbance experiments, a piece of Egeria was put in a beaker with 20 ml HCl solution for a certain time. Then absorbance was measured. In the experiment of the deflection method, a He-Ne laser was used as a light source of the probe beam. The probe beam was focused to a vicinity of Egeria in a culture dish by an objective lens. Deflection signals of the probe beam were monitored by a position sensor. The effects of acidic solutions and the distance between the probe beam and the leaf of Egeria were studied. Absorbance spectra of the HCl solutions with immersing time of the Egeria showed an absorbance peak around 320 nm. This suggested that some compounds were leaked from the Egeria into the HCl solution. Changes of absorbance and deflection signals with immersing time of Egeria were examined for the HCl concentrations with different pH. The changing trends with time of absorbance and deflection signals were similar, but the absorbance changing was delayed for about 2-3 hours. The deflection method was more sensitive than the absorption spectroscopy.
UV/VIS Spectroscopic Techniques and Related Methods

Studies of Chromium-6 Concentrations in Drinking Water Samples Coming From Three Different Sources of Water: Ground, Surface and Well Waters

Chromium exists in various oxidation states and there are two stable forms of chromium that can be found in the environment: trivalent (Cr^{3+} or Cr[III]) and hexavalent (Cr^{6+} or Cr[VI]) chromium. Both forms of chromium can be found naturally in the environment: in air, water or soil; but their existence is pH dependent. Cr(VI) is present in neutral or alkaline environment and can be converted to Cr(III) in acidic environment. Their toxicity level is also oxidation state dependent: Cr(III) is safe and an essential nutrient for humans, while Cr(VI) is known to be toxic and carcinogen.

In this study, the concentration of Cr(VI) in drinking water samples coming from three different types of sources were determined and compared. The sampling sites were within a 50 miles radius and their sources were ground water, surface water and well water. Additionally, the concentrations of Cr (VI) were analyzed from the influent and effluent of waste water treatment plants. A preliminary spectrophotometric method using 1,5-diphenylcarbazide as a reagent to react with Cr(VI) analyte was used and gave the following results: the method detection limit (MDL) ranged from 0.24 to 0.54 g/L, precision from 0.41 to 0.81% and accuracy from 94.9 to 106.0%. Cr(VI) was detected in all three sources of water. The majority of Cr(VI) concentrations in drinking water samples from ground water were found to be less than the MDL value. Cr(VI) concentrations in drinking water samples from surface waters were from 1.6 to 10.1 µg/L and from 0.6 to 6.6 µg/L in drinking water samples from well water.

Additional studies will be conducted to better understand the mapping of Cr(VI) concentrations, the origin of Cr(VI) and the correlation between these three sources of water within the 50 miles radius of sampling sites. The study will also include a development of on-site method to analyze Cr(VI) concentration as they are being collected.

Keywords: Contamination, Environmental/Water, UV-VIS Absorbance/Luminescence, Water
Application Code: Environmental
Methodology Code: UV/VIS
In the present investigation, we synthesize intrinsic and extrinsic MgO2 nanofilm on glass substrates using chemical dip method. MgO2 was doped with different dopant concentration (0%, 0.1%, 1% and 10%) of Neodymium and thin films of it was also made using chemical dip method. MgO2 nanofilms are synthesized using chemical dip method because this method is environmentally as well as economically friendly as compared to other methods. Structure and morphology of materials were determined using X-ray diffraction (XRD), Scanning electron microscope (SEM), UV-Visible spectroscopy (UV-Vis.) and particle thickness by ellipsometry spectroscopy. The XRD pattern showed that MgO2 nano material thin films and thin films of material doped with Neodymium were of amorphous in nature. Reflectance spectra of these samples were carried out in the range of 200-800nm at room temperature. Optical band studies show that the films are highly transparent and exhibit a direct band gap. Band gap of as prepared nanomaterials and doped nanomaterials is investigated using tauc plot. The bandgap of pure MgO2 thin films has been found to be lie in the range of 3.875 eV. The band gap of MgO2 thin film increases on doping with Neodymium. Thickness of the prepared nanomaterial MgO2 and with doped Neodymium thin films on glass substrate were carried out by ellipsometry spectroscopy. It was observed that the thickness of the thin films decreases on doping with Neodymium. SEM images of as prepared pure and rare earth metal Nd doped MgO2 nanoparticles on glass substrates were taken at different magnification and resolution. SEM images of pure MgO2 thin films shows grains are of uniform shape whereas on doping with Neodymium, sem shows irregular shape. This study would provides to us useful information about the changes in physical properties when we dope MgO2 with Neodymium.

Keywords: FTIR, UV-VIS Absorbance/Luminescence, X-ray Diffraction
Application Code: Nanotechnology
Methodology Code: UV/VIS
Reducing Data Redundancy in GC×GC–MS for Using the Unique Ion Filter

Using raw GC-MS data for chemometric analysis often results in more robust models, as peak detection and integration errors are avoided. One challenge is the enormity of variables especially when high data rate mass analyzers are used. In GC×GC-MS, fast detectors are a necessity due to the narrow second-dimension peaks observed. Consequently huge numbers of variables cannot be avoided in these experiments. This necessitates some variable reduction prior to chemometric analysis.

In an earlier report, we developed a data reduction tool termed unique ion filter (UIF), which provides an objective approach to feature reduction without compromising the multivariate nature of the raw data. This tool is applicable to GC/LC coupled with any multivariate data.

In our current work, we extend UIF’s variable reduction capabilities to comprehensive multidimensional separation with multivariate detection data, e.g. GC×GC–MS. GC×GC–MS data is essentially GC-MS data which can be folded into a 3D block using the modulation period and the data rate of the detector. Hence UIF can be applied to the raw data in GC-MS or GC×GC–MS modes.

In this study, we explore a new algorithm for um/z identification for GC×GC–MS data. We also compare the application of the UIF on raw data in GC-MS and GC×GC-MS modes for subsequent chemometric modeling to the use of peak tables obtained from commercial software as a data reduction strategy.

Abstract Text

Keywords: Chemometrics, Food Identification, Gas Chromatography/Mass Spectrometry, Other Hyphenated Tec
Application Code: General Interest
Methodology Code: Chemometrics
The National Institute of Standards and Technology (NIST) is a non-regulatory government agency within the Department of Commerce with the mission “To promote U.S. innovation and industrial competitiveness by advancing measurement science, standards, and technology in ways that enhance economic security and improve our quality of life”. This mission, guides the separations research at NIST towards improving measurement capabilities via several different routes. (1) A fundamental understanding of separation mechanisms is essential for understanding how to tune conditions to maximize separations. A good example of this is the work performed by Sander and Wise to study and elucidate shape selectivity, which is useful for the separation of geometrically constrained isomers. (2) The application of this fundamental knowledge can be used for the design of stationary phases tailored specific separation tasks. (3) The application of cutting edge separation technologies, such as multi-dimensional separations, is studied to determine the applicability of the new techniques to the determination of analytes in complex matrices. Lessons learned from stationary phase synthesis, column characterization, and multidimensional chromatography will be discussed.

Keywords:  
HPLC, Reference Material, Separation Sciences

Application Code:  
General Interest

Methodology Code:  
Liquid Chromatography
The presentation will describe the development of chromatographic methods that are part of an analytical toolbox that can be utilized for the advancement of vaccine candidates from discovery through commercialization. The vaccine antigens in this case consist of different constructs including protein antigens by themselves, peptides or small molecules conjugated to carrier proteins, or polysaccharides conjugated to a carrier proteins. As part of development, traditional and novel column technologies were evaluated for the characterization of size, charge, purity, and titer as well as other attributes that may impact the immunogenicity of the vaccine candidate. In many cases it is also desirable to discriminate amongst several different antigens included in a drug product that also may include an adjuvant. Since there is quite a bit of diversity in the molecular properties for the vaccines in development, there is currently not a one size fits all approach for vaccines as may be the case for many monoclonal antibodies in development. This presentation will focus on some of the highlights from our recent chromatographic method development activities to progress the vaccine portfolio which utilizes some new instrumentation and novel column technologies.

Keywords: HPLC, HPLC Columns, HPLC Detection, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Food allergy is a major public health concern that affects up to 8% of children and up to 2% of the adult population. To protect the allergic consumer with accurate food allergen labeling, reliable analytical methods are required for allergen detection and quantification. These methods must be effective in spite of food processing-induced changes in the biophysical and immunological properties of multiple allergen proteins in a food matrix. The present work is focused on the application of analytical techniques used to decipher the chemistry that underlies allergen protein interactions in thermally processed foods.

Combining enhanced solubilization and sophisticated protein fractionation with high resolution mass spectrometry (MS), enables a peptide-specific view of changes in allergen proteins using variable sample extraction and processing conditions. Size-selective GELFrEE fraction is coupled with liquid chromatography (LC)-MS in a multidimensional platform developed for comprehensive global mapping of the [i]A. hypogaea[/i] proteome. Visualization of allergen peptide molecular distributions by GELFrEE-LC-MS/MS, moreover, aids in the identification of protein rearrangements, modifications, and structural isoforms in peanuts. Expanding upon this work, a comprehensive LC-MS/MS platform is applied for simultaneous multi-allergen detection in thermally-processed food samples incurred with milk, egg, and peanut. Relative quantification of allergens in protein lysates was accomplished by label-free spectral feature (MS1) LC-MS/MS methodologies to identify differentially-abundant peptides resulting from thermally-induced protein modifications. A combined analytical approach incorporating global proteomic screening with qualitative gel-based analyses promotes an advanced understanding of fundamental changes in allergen proteins induced by food processing chemistry, thereby improving the performance of detection methods for allergens in complex food systems.

Keywords: Food Safety, Liquid Chromatography, Mass Spectrometry, Proteomics
Application Code: Food Safety
Methodology Code: Liquid Chromatography/Mass Spectrometry
Complex samples with multiple compound classes present, compound similarity (isomers), and a wide range of analyte concentrations all present a challenge to conventional gas chromatographic (GC) separation techniques. One powerful technique for the analysis of complex samples is comprehensive two-dimensional gas chromatography (GC×GC). Another is a recently developed vacuum UV (VUV) detector that can be coupled to a GC and is capable of collecting full spectral scans from 125 to 240 nm on the millisecond time scale with less than 1 nm spectral resolution. VUV spectra can be compared to reference spectra, although relatively few are available, compared to known experimental standards, or predicted by molecular modeling to confirm structure. The VUV detector is capable of providing compound quantification as well. The VUV has shown promise in differentiating chemical classes, some of which include: alcohols, aldehydes, esters, acids, glycol ethers, amines, thiols, sulfides, chlorinated aliphatics. Use of specific spectral ranges can aid selective detection of certain compounds or enhance detector sensitivity. The combination of the new VUV detector with a GC×GC instrument may provide the next step towards ‘routine’ analysis of complex samples in regards to class separation and quantification. In this presentation, the coupling of a VUV detector with a reverse fill/flush flow modulation (RFF) comprehensive GC×GC instrument for selected industrial applications will be presented.

Keywords: Gas Chromatography, GC Detectors, Petrochemical, Separation Sciences

Application Code: Fuels, Energy and Petrochemical

Methodology Code: Separation Sciences
Metallic nanostructures with their ability of confining and enhancing incident light offer unique possibilities for manipulating light at the nanoscale. [1] Plasmons can efficiently capture incident light and focus it to nanometer sized hotspots which can enhance electronic and vibrational excitations in nearby structures. In this talk, I will discuss how recently discovered fundamental plasmonics effects, plasmonic Fano resonances and quantum plasmonics, can be exploited for surface enhanced spectroscopies. Plasmonic Fano resonances with their characteristic narrow and asymmetric line shapes can provide very large field enhancements making such modes particularly useful in LSPR sensing and surface enhanced spectroscopies such as SERS,[2] Four-wave mixing,[3] and SECARS.[4] Quantum mechanical effects such as electron tunneling[5] and hot carrier generation[6] on the surface of a plasmonic nanostructure can induce charge transfer into adsorbed molecules and change their cross sections.

References


Keywords: Nanotechnology, Raman, Spectroscopy, Surface Analysis
Application Code: Materials Science
Methodology Code: Vibrational Spectroscopy
Biological TERS: Instrumentation Development and Applications

Refractive Index Monitoring at the Nanoscale

The near field enhancement in tip-enhanced Raman spectroscopy (TERS) implicitly relies on the tip-sample interaction as part of the tip's surface plasmon resonance. While this resonance can be considered to be spectrally large as compared to the Raman signature, the exact peak position does significantly depend on the dielectric permittivity of the surrounding media and in particular the local refractive index of the sample environment as part of this media.

Our initial experiments were meant to demonstrate the chemical and structural integrity of ferroelectric nanostructures and while we were indeed able to achieve a spatial resolution below 5 nm, the back-scattered light contained a strong tip signature that was superimposed to a relatively weak Raman signal from the sample. Approximating the surface plasmon resonance by a Lorentzian line shape paved the way to discriminating tip again sample contributions.

We thus demonstrate for the initial case of inorganic nanostructures how residual fluorescence from the apex of the gold tip can be used to determine the relative shift of the surface plasmon resonance which has three immediate implications: first, the quality factor of TERS tips depends strongly on the sample so there is no ‘perfect’ TERS tip to cater for all samples and second, the peak intensities of the Raman spectrum become a function of the envelope of the enhancing surface plasmon, which depends again on the position on the sample. Last but not least, we demonstrate how to extract local refractive index variations of the sample with a sensitivity of better than 10 percent relative change, which enables detection schemes for local phase transformations, variations of oxidation states or at least a direct optical signature from the tip, which is modulated through the sample in cases where the Raman signature might be otherwise undetectable.

Keywords: Electrochemistry, Infrared and Raman, Surface Enhanced Raman, Vibrational Spectroscopy
Application Code: Materials Science
Methodology Code: Vibrational Spectroscopy
Intra- and intermolecular coupling and dynamics determines chemical reaction, charge transfer, biological, and catalytic activity of molecules. Spectroscopic access has continued to challenge vibrational photon-echo and its multidimensional spectroscopy implementations to probe the homogeneous system response. In contrast, tip-enhanced Raman spectroscopy (TERS) can intrinsically probe at the homogeneous sample size limit by virtue of its nano-meter spatial resolution and single molecule sensitivity. We perform systematic TERS measurements at cryogenic and variable temperature of dilute malachite green monolayer physisorbed on a template stripped gold surface. The experiments reveal details of the molecular dynamics over a wide range from few 100's fs intramolecular dephasing to seconds for low temperature structural reorientations. Line narrowing, splitting, and the appearance of slow mode hopping with decreasing temperature shows that the inhomogeneous broadening at room temperature is characterized by high-frequency fluctuations of molecular orientation and/or structure. The temperature dependence allows for the separation of a temperature independent dephasing process from an Arrhenius-type activated process with a few 100 cm\(^{-1}\) activation energy indicative of vibrational stretch and combination band coupling to low energy intramolecular bending and torsional modes. Corresponding coupling constant and lifetime of the low frequency modes are consistent with literature values from related photon echo experiments. In the homogeneous sample size limit we observe spectral fluctuations that can be interpreted as discrete orientational changes of the molecules on the surface. The correlation of spectral fluctuations of coupled normal modes provide additional evidence of a single molecule response.

**Keywords:** Molecular Spectroscopy, Raman, Ultra Fast Spectroscopy, Vibrational Spectroscopy

**Application Code:** Materials Science

**Methodology Code:** Vibrational Spectroscopy
During the last few years, the study of ultrahigh vacuum tip-enhanced Raman spectroscopy (UHV-TERS) has been raised to an unprecedented level. By using ex-situ laser focusing and Raman collection optics without compromising UHV, multiple vibrational modes for molecular adsorbates on a solid surface have been resolved in TER spectra obtained concurrently with sub-nm resolution Scanning tunneling Microscopy (STM) imaging. In addition, the adsorbate-substrate interactions can be studied. With liquid helium cooling sample, molecules no longer diffuse on the surfaces. New vibrational characteristics of TER spectra can be obtained in R6G / Ag(111) system at 19K. Theoretical analysis of vibrational modes allowed interrogation of the interaction between rhodamine 6G and the Ag(111) surface. Further a substantial progress in TERS application is the integration of ultrafast spectroscopy with TERS. TERS using pulsed excitation in both atmosphere and UHV has been demonstrated. And our results suggest that the UHV environment mediates signal decay. This approach provides the tools to understand the dynamical processes such as – plasmon driven charge transfer with unprecedented temporal and spatial resolution.

Keywords: Nanotechnology, Raman, Spectroscopy
Application Code: Nanotechnology
Methodology Code: Vibrational Spectroscopy
Tip-enhanced Raman spectroscopy (TERS) is a powerful tool for probing the surface of amyloid fibrils with a nanometer spatial resolution. Here we report TERS maps of individual insulin fibrils and a short peptide (LVEALYL) microcrystal, which is expected to mimic the fibril core. Microcrystal TERS spectra measured at different spots are similar to each other and very close to the conventional Raman spectrum. In contrast, TERS spectra of insulin fibrils vary significantly from spot to spot and show very little resemblance with a conventional Raman spectrum. In addition, TERS signal obtained from fibrils is significantly stronger than that of the microcrystal. A hypothetical mechanism of tip enhancement resulting in different Raman spectra acquired for fibrils and microcrystal will be discussed.

Keywords: Imaging, Raman
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
### Abstract Title

**Electronic Noise of the α-Hemolysin Latch Sensing Zone Reflects the Stability of DNA Duplexes Confined Within a Nanopore**

### Abstract Text

The latch region of the wild-type protein pore alpha hemolysin constitutes a sensing zone for individual abasic sites in double-stranded DNA (dsDNA). The presence of an abasic site within a DNA duplex, electrophoretically captured in the α-HL vestibule and positioned at the latch region, can be detected based on the current blockage prior to duplex unzipping. Variations in blockage current were investigated as a function of temperature and KCl concentration to understand the origin of the current signature and to optimize conditions for identifying the base modification. Optimal resolution for detecting the presence of a furan in the latch region is achieved at lower KCl concentrations, where the noise in the measured blockage current is significantly lower. The noise associated with the blockage current also depends on the stability of the duplex (as measured from the melting temperature), where a greater noise in the measured blockage current is observed for less stable duplexes.

### Keywords

- Bioanalytical
- Biomedical
- Biosensors

### Application Code

- Bioanalytical

### Methodology Code

- Sensors
Abstract Text

Single pores have been used as a basis for the detection of single molecules, viruses, particles and cells using the principle of the Coulter counter approach. Each object passing through a pore causes a transient change of the system resistance whose amplitude and duration give information on physical and sometimes even chemical properties of the object. The Coulter counter technique typically involves recording passage of many particles so that appropriate statistics of the translocation time and the pulse amplitude can be obtained. In case however of an unknown and potentially diluted mixture, a different approach might have to be taken. Instead of statistics on an ensemble of particles/molecules, statistics based on one particle studied many times is needed. In the presentation, we will discuss resistive-pulse experiments with polystyrene particles whose transport through pores is controlled by modulating the driving voltage during the process of translocation. Balancing all forces acting on single particles allowed us to observe random walk in a form of ion current fluctuations in time, and diffusion coefficient of individual particles was determined based on variance of their local diffusion velocities. The developed approach is applicable to particles of different sizes, does not require fluorescence labeling or tracking, and is entirely based on ion current recordings. Analysis of particles from mixture will be presented as well. The same technique was used to trap single particles in the pore for tunable time between tens of milliseconds to 40 seconds.

Keywords: Biosensors, Biotechnology, Materials Science, Membrane

Application Code: Materials Science

Methodology Code: Physical Measurements
Chemical Noise
Stochastic Signals in Electrochemical Nanofluidic Devices

Lithography-based microfabrication allows creating fluidic devices with well-defined dimensions below 100 nm. We have employed this capability to create nanoscale thin-layer cells and interfacing them to microfluidic channels, thus simultaneously allowing highly efficient redox cycling and independent control of advective mass transport. The electrochemical response of the devices is characterized by stochastic fluctuations associated with the small number of analyte molecules present in the femtoliter detection volume coupled with the random nature of Brownian motion. Analysis of these fluctuations permits extracting information which is otherwise mostly inaccessible, as illustrated by our use of number fluctuations to measure record-low flow rates of order pL/min in nanochannels as well as in-situ potential-dependent adsorption of redox species. The high degree of amplification further allows detecting single redox-active molecules and quantitatively studying the statistics of their individual Brownian trajectories.

Keywords: Electrochemistry, Lab-on-a-Chip/Microfluidics, Method Development, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Electrochemistry
Chemical Noise

Effect of Chromatographic Sampling Width on Chemical Noise, Sensitivity, and Detection-Stage Requirements in Hyphenated Analytical Systems

Liquid and gas chromatography have been used as up-front separation approaches for a variety analytical techniques in so-called hyphenated measurement systems. Examples are LC-MS and GCxGC, widely used in the analysis of complex mixtures. In these systems, a segment of the chromatogram is passed to a subsequent device for further analysis. While up-front separation is meant to simplify analysis and/or achieve additional analytical resolution, complications can arise from the segmentation itself. Because component peaks are split between, or among, chromatographic segments, the absolute and relative concentrations of compounds within a segment are not representative of the mixture. Here, we assess how chemical noise, component response factor, and sample load and complexity depend on the width of the passed-on segment relative to a chromatographic peak. Computer simulation studies demonstrate that, for complex mixtures in which the distribution of component concentrations is taken to be lognormal, chromatographic-segment width is a critical parameter that affects the number and distribution of compounds detectable in complex mixtures. We find that chemical noise is greater for short segments than for the whole sample. Additionally, as segment width decreases toward the peak width, the total measured response of each component also decreases, while the chromatographic resolution improves. The resolving power of the subsequent stage and other factors have a large effect on the selection of the optimum segment width.

Keywords: Data Analysis, Gas Chromatography/Mass Spectrometry, Liquid Chromatography/Mass Spectroscopy
Application Code: Other
Methodology Code: Computers, Modeling and Simulation
Interfacial boundary layer scattering at atomic-scale junction (ASJ) surfaces is a powerful mechanism to sense chemisorption, particularly of Lewis bases, and metallic nanowires are well-matched to the problem of chemical detection of mass-limited samples, such as those handled in integrated microfluidic structures. ASJs at the narrowest constriction are one or a few atoms in the transverse dimension. At open circuit, current conduction in the ASJ is dominated by ballistic transport, and ASJs are exceptionally sensitive to adsorption-induced alterations in electron scattering. Furthermore, fluctuations in the population of surface adsorbates produces chemical noise in the conductance that can be studied by fluctuation spectroscopy of the power spectral density (PSD). The PSD exhibits two main frequency regions: $1/\langle i \rangle$ noise originating from resistance fluctuations of the junction itself, and a Lorentzian noise component arising from molecular adsorption/desorption fluctuations at higher frequencies, the latter carrying information about the kinetics of the adsorption/desorption process. Furthermore, studying these phenomena under electrochemical potential control provides a reliable method of regulating the size and stability of these nanojunctions. In general, more anodic potentials decrease junction stability and increase the rate at which conductance decays. Electrochemical potential also plays a significant role in determining adsorption-desorption kinetics of surface adsorbates at steady-state at Au-Ag-Au ASJs, as revealed through fluctuation spectroscopy. Average cutoff frequencies increase at more anodic potentials, as does the width of the cutoff frequency distribution.
Ambient sample introduction systems are for organic compounds are widespread and have increased the capabilities of various techniques. Elemental and isotope analysis by ICP-MS still suffers from the fact that ambient aerosols cannot be introduced into such instruments. Little amounts of oxygen extinguish the Plasma. Therefore, a gas exchange device (GED-III) was tested for the online analysis of various gas species (airborne and synthetically generated). A particle generation process developed by J-Science was tested to investigate the detection efficiency by ICPMS spectrometry. The optimization procedure and few selected applications will be reported.

Keywords: ICP-MS, Laser, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Inductively-coupled plasma mass spectrometry (ICP-MS) is a vanguard analytical technique, however, new instrumental approaches can unlock even more significant capabilities. Here, a new type of mass analyzer known as the distance-of-flight mass spectrometer (DOFMS) will be evaluated for use in ICP-MS. The DOFMS concept is best explained by comparison with traditional time-of-flight mass spectrometry (TOFMS). TOFMS measures the mass-to-charge (m/z) of an ion by imparting the same energy to all ions and then measuring the time required for each m/z to traverse a distance and arrive at a single detector. In contrast, DOFMS measures the m/z of an ion by measuring the distance each ion travels during a set time period. Simply put, ions of lower m/z traveling longer distances than ions of greater m/z, and their m/z can be determined based upon location.

The DOFMS strategy offers a number of significant benefits for ICP-MS. Like TOFMS, DOFMS is architecturally simple, offers very rapid spectral generation rates, and is capable of simultaneous multielemental analysis. However, DOFMS is able to employ new solid-state array ion detectors to great advantage, providing greater detection efficiency and dynamic range than typical TOFMS, and obviating the need for fast electronics. As important, the DOFMS strategy permits new experiments to be performed. For example, DOFMS focusing strategies can be employed in a typical TOFMS experiment to improve mass resolution over a limited window of the atomic mass spectrum. This technique, known as Zoom-TOFMS, is able to increase mass resolution while simultaneously increasing the S/N of the mass spectrum. Moreover, because TOFMS and DOFMS share a common architecture, a single instrument is able to switch between TOFMS, DOFMS, and Zoom-TOFMS experiments as required. The theory of operation and experimental advantages of DOFMS will be discussed, and the analytical performance of this new type of mass spectrometer will be described.
Laser ablation sampling provides elemental and isotopic analysis, with minimal or no sample preparation, no consumables, and rapid turn-around time. The ablated mass is analyzed by optical emission from the induced plasma (LIBS – Laser-Induced Breakdown Spectroscopy and LAMIS – Laser Ablation Molecular isotopic Spectroscopy) and/or from the aerosol (particles) that is transported into a secondary source like the ICP (Inductively Coupled Plasma). Every element on the periodic chart, from H to Pu can be analyzed using these technologies.

LIBS has experienced a revival in the past ten years, mainly due to the benefits of no gases, simpler system components and the ability for stand-off applications. With new stable laser sources and intensified detectors, LIBS applications are increasing daily and rugged commercial instruments are available. Chemometric data processing is driving applications further. LIBS has become a workhorse technology for many routine applications; several of which will be mentioned in this talk. By expanding the capabilities of classical LIBS to emphasize the measurement of molecular emission spectra in addition to elemental, LAMIS provides the ability to measure all elements and their isotopes, especially light elements like Li, Be, C, N, O. We developed LAMIS to date by establishing its ability to measure B, C, H, D, Sr and other isotopes. We demonstrated low percent levels for sensitivity and have experimental plans to meet ppm levels. For some isotopes, we achieved < 0.1% precision. The talk will describe the isotope work that has been reported in LIBS plasmas and show how LAMIS expands those capabilities.

Keywords: Atomic Emission Spectroscopy, Atomic Spectroscopy, Elemental Analysis
Application Code: Other
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Oceanography is rapidly moving from a sampling science, where measurements are made aboard ship on specimens retrieved from the water column or seafloor, to a sensing science, where instrument systems directly collect measurements in situ, potentially for long periods of time. However, appropriate sensor technologies are required to carry out sensing science, and those for chemical measurements are not as advanced as in other disciplines. Laser-induced breakdown spectroscopy (LIBS) is an existing in situ technique that can potentially address this shortcoming by extending it to the deep ocean aqueous environment. LIBS uses a pulsed laser and gated detector to measure atomic emission spectra from a laser-induced plasma that forms following laser ablation of the sample. A long-range goal of work being carried out jointly by our group at The University of South Carolina and Woods Hole Oceanographic Institute is deploying a LIBS system on Alvin or other deep-ocean submersibles to measure the elemental composition of deep-ocean hydrothermal vent fluids. This talk will include a review of previous work at USC measuring ppm levels of alkali and alkaline metal elements at pressures up to 3×10E7 Pa (~2800 m water depth equivalent), and studies of matrix interactions between different elements. In addition, new work using O and H as internal standards to improve measurement precision will be presented and studies related to the effect of suspended particulates will also be presented. In other applications of LIBS in inaccessible places, planetary applications of LIBS are being investigated. To this end a miniature spatial heterodyne LIBS spectrometer has been developed and used for the first time for standoff LIBS. This instrument uses a 10 mm diffraction grating for light collection at distances up to 20 meters, corresponding to a collection solid angle of less than one microsteradian.

Keywords: Atomic Emission Spectroscopy, Atomic Spectroscopy, Elemental Analysis, Laser
Application Code: Other
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Emerging Trends in Plasma Spectrochemistry

The Expanding Role of Glow Discharges in Analytical Science

In recent years, glow discharges have assumed broader and perhaps even greater importance, especially in the form of atmospheric-pressure glows. At atmospheric pressure, the customary spatial structure of the discharge is conserved, but the dimensions are collapsed, the current-voltage behavior is altered, and the distribution of species in the plasma is changed. As a result, the applications of the glow have broadened and it has assumed even greater importance analytically. In this presentation, several examples of novel glow discharges will be described and evaluated. One, the solution-cathode glow discharge (SCGD) is sustained in the open atmosphere directly on the surface of a solution to be analyzed. Material from the solution is then volatilized, atomized, and excited, to yield a source useful for the direct elemental analysis of sample solutions. Even though the SCGD consumes little power (~75W), needs no gas supply, nebulizer or spray chamber, and emits simple spectra, it yields detection limits that rival those of the inductively coupled plasma. In another incarnation, a glow discharge in helium can be used as a source for ambient desorption/ionization mass spectrometry. In this application, sample material need not be introduced into the discharge itself, but only into the effluent from the discharge cell. Also, because the Flowing Atmospheric-Pressure Afterglow (FAPA) heats the flowing helium to selectable temperatures nearing 300°C, species can be desorbed directly from the surface of a solid sample, and ionized in the helium stream, to yield mass spectra largely of the parent ion or its protonated counterpart. Here, a recent version of the FAPA source will be introduced and its application featured. Finally, a more conventional glow will be evaluated. Operated at reduced pressure and powered by radiofrequency energy, this pulsed discharge enables the three-dimensional elemental composition of conductive or non-conductive solid samples to be determined.

Keywords: Atomic Spectroscopy, Instrumentation, Mass Spectrometry, Method Development
Application Code: General Interest
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Despite the central role of soil microbial communities in the global carbon (C) cycle, very little is known about soil microbial community composition and even less about their metabolic pathways. High throughput sequencing technologies have recently created unprecedented access to the biodiversity of soil microorganisms, allowing characterization of the microbial mechanisms regulating soil C cycling. We coupled soil metagenomic and environmental analyses to identify the “core” C-cycling sequences that are consistently prevalent in soil samples from a fertilized prairie (FP) biofuel cropping system. Of 226,887 sequences associated with known enzymes involved in the synthesis, metabolism, and transports of carbohydrates (the CAZy database), 911 sequences were identified to be consistently prevalent across four replicate soil metagenomes. This core metagenome was functionally and taxonomically diverse, representing five major enzyme classes and 107 enzyme families within the CAZy database and numerous bacteria and fungal phyla. Despite comprising only 0.4% of the cumulative CAZy-associated FP metagenome, the core was found to be functionally representative of the cumulative whole soil metagenome, suggesting that these sequences may represent key C cycling genes in this soil. Comparing the FP CAZy-associated core to other soil metagenomes revealed that the sequences in the FP core are present in multiple other soils, and most similar to soils sharing geographic proximity. Several co-occurring core sequences were identified in functional modules representing distinct metabolic processes in starch and sugar metabolism. In soil ecosystems, where high diversity remains to be a key challenge for metagenomic investigations, these core genes represent a subset of critical functions necessary for carbohydrate metabolism, which can now be targeted to evaluate key carbon cycling organisms and pathways that are relevant to agricultural systems and biofuel reactors.

Keywords: Bioinformatics, Carbohydrates, Environmental/Soils, Enzyme Assays
Application Code: Environmental
Methodology Code: Other
Cyanobacteria are found in most types of illuminated environments and the global biomass of these photosynthetic microbes has been estimated to be $\sim 3 \times 10^{14}$ g carbon (C). Although their key role in global C and nitrogen (N) cycling has been widely accepted and significant efforts to advance our understanding of this ecologically important phylum have been made, very little is known about how Cyanobacteria affect and respond to environmental changes. This knowledge gap is mostly due to the fact that, until recently, approaches facilitating in situ studies of complex microbial systems were rather limited. With the advent of advanced cultivation-independent techniques (e.g. metagenomics, metatranscriptomics, metaproteomics) it is now possible to study microbial communities, their metabolic potential, and their metabolic activity without the need to isolate and grow individual community members or even the complex consortia in the laboratory.

In the project presented here, we took advantage of a suite of unique Omics capabilities available at DOE’s Joint Genome Institute and Environmental Molecular Science Laboratory to obtain a multi-scalar (from molecule to cell to population to ecosystem) understanding of photosynthetic consortia that contain several distinct Cyanobacteria and several heterotrophic microorganisms and to determine how the community and its individual community members affect the C and N cycle. In addition, our team utilizes the diverse Omics data generated during this project to develop computational and experimental approaches that will facilitate the isolation of pure cultures of microorganisms that are recalcitrant to current isolation and cultivation techniques. Recent findings will be presented.
The outcome of a viral infection is influenced by the balance between host defense mechanisms and virus modulation of cellular pathways. We have integrated genomics with proteomics and molecular virology to identify novel host defense factors that can provide new targets for antiviral therapeutics. Currently, most antiviral drugs target specific viral proteins. Consequently, they often work for only one virus and their efficacy can be compromised by the rapid evolution of resistant variants. There is a need for the identification of host proteins with broad-spectrum antiviral functions, which provide effective targets for therapeutic treatments that limit the evolution of viral resistance. Here, we report the discovery that all seven human sirtuins have antiviral properties. Sirtuins are a family of ubiquitously expressed and evolutionarily conserved NAD-dependent deacylases/mono-ADP ribosyltransferases that regulate numerous cellular and organismal functions, including transcription, metabolism, and longevity. Our results show that sirtuins exhibit defensive properties against a range of DNA and RNA viruses. Furthermore, we show that the sirtuin defense functions are evolutionarily conserved, as CobB, the sirtuin homologue in E. coli, protects against bacteriophages. Altogether, our findings establish sirtuins as broad-spectrum and evolutionarily conserved components of the immune defense system, providing a framework for elucidating a new set of host cell defense mechanisms and developing sirtuin modulators with antiviral activity.
The availability of complete microbial genome sequences for cultured organisms as well as more complex environmental samples has enabled systems biology interrogation of microbial communities by integrating genomic, transcriptomic, proteomic and metabolic information. Our current work seeks to develop and demonstrate advanced “shotgun” mass spectrometry techniques for the comprehensive characterization of complex microbial proteomes. The goal of this research is to enable integration of metagenomics and metaproteomics datasets for a detailed glimpse into the functional state and metabolic activities of microbial systems, spanning the range from single isolates to more complex consortia.

The proteome approach is based on multidimensional liquid chromatography interfaced on-line with tandem mass spectrometry. A variety of mass analyzers are employed, ranging from quadrupole ion traps (high throughput) to Orbitraps (high performance, accurate mass measurements). We have focused on integrated experimental/bioinformatic approaches for both qualitative and quantitative proteome measurements. We have optimized a detergent based proteome extraction method which can be combined with high performance LTQ-Orbitrap-Velos measurements to investigate natural microbial communities, such as those involved in carbon cycling in terrestrial (soils, sediments) as well as aqueous (acid mine drainage, deep sea methane seeps) systems. We have extended these MS-based techniques to characterize a variety of microbial species that are involved in bioremediation, bioenergy, human microbiome health, and natural environmental communities.

Research support was provided by the U.S. DOE, Office of Biological and Environmental Research. Oak Ridge National Laboratory is managed and operated by the University of Tennessee-Battelle, L.L.C., for the U.S. Department of Energy.

**Keywords:** Bioinformatics, Genomics, Mass Spectrometry, Proteomics

**Application Code:** Genomics, Proteomics and Other ’Omsics

**Methodology Code:** Mass Spectrometry
Tumors are well known to have poor mass transport, resulting in nutrient-poor regions with high waste concentrations. As a result, the tumor microenvironment can trigger cell survival mechanisms that inhibit apoptosis, a key mechanism for many chemotherapeutic compounds. In an effort to isolate the causes for increased drug resistance in tumor cells, we have developed a "hypoxia chip" to induce hypoxia in cells. This system is based on our low-shear culture design and can accommodate multiple culture types as well as on-chip spectroscopic oxygen detectors. In experiments with hypoxic prostate cancer cells, we observed a marked increase in drug resistance when compared with normoxic controls. We detected apoptosis via fluorescence assays for mitochondrial leakage and membrane rearrangement.

We have shown that short (<60 min) periods of hypoxia are sufficient to impart enhanced drug resistance in cells, and have shown that prostate cells continue to follow a dose-response characteristic curve, albeit at higher concentrations. This work has implications not only in drug testing, but also in the basic mechanisms of cancer cell drug resistance.

Keywords: Bioanalytical, Biological Samples, Biomedical, Imaging
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
In recent years, there are significant interests in the manipulation of cells using electric, magnetic, acoustic and/or flow fields for separation, characterization, actuation, and assembly of particles. In this talk, a brief overview of electric field driven cell manipulation techniques will be provided. Existing models used to study electric field driven cell manipulation will be highlighted along with their strengths and shortcomings in microdevice. The recently developed hybrid immersed interface-immersed boundary method will be introduced to study electric field driven particle assembly where both electric and hydrodynamic forces are calculated with interface-resolved approach instead of commonly used point-particle method. In this hybrid model, the immersed interface method is employed to capture the physics of electrostatics in a fluid media with suspending cells, while the immersed boundary method is used to study hydrodynamics with rigid or flexible immersed boundaries. Moreover, the Maxwell stress tensor is used to calculate the electrostatic force acting on particles by considering the physical effect of particles in the computational domain. Thus, this method eliminates the approximations used in point dipole methods for calculating forces. Finally, electric field driven cell motions and related fluid flow phenomena will be presented for charged, uncharged and bipolar cells. The self-assembly of cells will be demonstrated for similar and dissimilar types of cells using applied electric fields.

Keywords: Biomedical, Lab-on-a-Chip/Microfluidics, Nanotechnology
Application Code: Biomedical
Methodology Code: Computers, Modeling and Simulation
## Session Title
Microfluidics Meets Cell Analysis

## Abstract Title
Isolation, Manipulation, and Analysis of Single Cells

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### Abstract Text
The ability to correlate single-cell genetic and protein expression information to cellular phenotypes will provide the kind of detailed insight into human physiology and disease pathways that is not possible to infer from bulk cell analysis. This presentation describes a set of single-cell isolation, manipulation, and analysis techniques that we have developed in our lab, including a rare-cell isolation platform we called eDAR, the active manipulation and trapping of single cells using bipolar electrodes and dielectrophoresis, the analysis of single-cell gene expression with digital PCR on a SD chip, and the quantification of expressions of a large set of proteins at the single-cell level.

### Keywords
- Bioanalytical
- Biomedical

### Application Code
Biomedical

### Methodology Code
Microfluidics/Lab-on-a-Chip
Microfluidics Meets Cell Analysis

Electrochemical Detection in Microchip Electrophoresis: Application to the Determination of Nitrogen Reactive Species in Cells and Its Monitoring

Reactive Nitrogen Species (RNS), and in special Nitric Oxide are involved in many biological processes. It has been demonstrated that Nitric Oxide plays an essential role in inflammatory pathway, blood pressure control, cell signaling, neurotransmission, gene expression, coagulation, among other. The study of RNS is difficult due to the reduced concentrations and short lifetime of these species. We have developed strategies to monitor RNS in model cells, such as RAW 264.7 macrophages and lymphocytes, using microchip electrophoresis coupled with amperometric detection. Microchips were built in poly(dimethylsiloxane) and designed to contain one or two separation channels. Samples were injected using gated injection. Calibration procedures for determination of Nitric Oxide can be performed using NO donors compounds, such as NONOates salts. We also demonstrate the viability of cell monitoring using capacitively coupled contactless conductivity detection.

Keywords: Bioanalytical, Capillary Electrophoresis, Electrochemistry, Lab-on-a-Chip/Microfluidics

Application Code: Bioanalytical

Methodology Code: Microfluidics/Lab-on-a-Chip
Microfluidics Meets Cell Analysis

Rapid Single Cell Analysis on Integrated Microfluidic Devices

In the past few years microfluidic devices have begun to change the way cellular analysis is performed. This is because of their ability to integrate cell transport, manipulation, stimulation, and lysis with the separation and detection of the analytes released by the lysis of such cells. The high throughput analysis of single cells is of interest because of their heterogeneous behavior under a variety of conditions. A better understanding of this heterogeneity would improve our understanding of neurodegenerative diseases. Microfluidics might also be employed in the development of early detection systems for these diseases. We have recently developed a microfluidic device architecture to study the expression of nitric oxide (NO) in Jurkat cells. Our experiments compared the production of NO in native and lipopolysaccharide stimulated cells. The cells were labeled with fluorogenic dyes including 4-amino-5-methylamino-2',7'-difluorofluorescein diacetate (DAF-FM DA) which, when hydrolyzed, reacts with NO within the cell. On the microfluidic device, the labeled cells were transported to an intersection where they encountered high voltage that generated cell lysis, injection, and electrophoretic separation prior to laser induced fluorescence (LIF) detection. In all, more than 1000 cells were analyzed, and the average results compared nicely to recently published bulk cell studies performed for the analysis of NO.

Keywords: Bioanalytical, Biological Samples, Capillary Electrophoresis, Lab-on-a-Chip/Microfluidics
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
The discovery and identification of a fiber as a particular polymer (e.g., acrylic, cotton, nylon, polyester) may not, of itself, provide much support for a forensic investigation. The probative value of fibers found at a crime scene depends on their uniqueness relative to the fiber background in the absence of the crime. What is required is information that makes trace evidence more specific and discriminating. Based on a collection of 3,000 textile fibers, we have developed a web-based system for storage of microscopy measurements, physical characteristics, and spectral data that facilitates use of multivariate statistics for assessing discrimination and classification of fibers for forensic comparison. Following derivative processing, baseline correction, and normalization, UV/visible and fluorescence spectra from 482 fibers were classified using principal component (PC) and linear discriminant analysis (LDA) in each color and polymer group. The highest discrimination power was obtained from UV/visible spectra, which were correctly classified 89.50% of the time.

The transfer of multivariate classification models between laboratories can save time and resources in forensic analyses. Using UV/visible spectra of 12 blue acrylic fibers, collected at five different laboratories, transferability of models was investigated using PCA-LDA and partial least squares-discriminant analysis (PLS-DA). An average classification of 88.06% was found after training the PLS-DA models using data collected at four laboratories and using the information collected at the fifth laboratory as an external test set. For comparison, intra-laboratory studies carried out using PCA-LDA produced an average classification accuracy of 98.33%. These results suggest limitations to the transferability of classification models between laboratories. Researchers are advised to use caution and to follow the traditional adage of "Trust, but verify."

Keywords: Chemometrics, Forensics, UV-VIS Absorbance/Luminescence
Application Code: Homeland Security/Forensics
Methodology Code: Chemometrics
Chemical analysis of fire debris, as implemented in most US forensic laboratories, involves a relatively easy sample preparation followed by gas chromatography-mass spectrometry with electron ionization. While the analytical methodology is simple, data analysis is more complex and requires the forensic analyst to use visual pattern recognition, coupled with target compound analysis to arrive at a subjective assessment of a sample as positive or negative for the presence of ignitable liquid residue. Although the characteristics of the ignitable liquid classes, as defined by the ASTM E1618 standard method (i.e., gasoline, petroleum distillate, etc.), can assist the analyst in recognizing the presence of ignitable liquid residue in fire debris, the complexity of the sample may be greatly enhanced by the presence of pyrolysis products. Standard classifiers have been used to assign fire debris samples as positive or negative for the presence of ignitable liquid residue, and for class assignment. The performance of classifiers based on linear and quadratic discriminant analysis (LDA and QDA respectively) and soft independent modeling of class analogy (SIMCA) will be discussed. Application of support vector machines will also be discussed along with classification with support vector machines and the incorporation of a Bayesian approach with a verbal scale, which can assist the analyst in decision making and presentation of the results in a legal context.

This project was supported in part by Award No. 2009-DN-BX-K227 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication/program/exhibition are those of the author(s) and do not necessarily reflect those of the Department of Justice.
**Abstract Text**

The FBI has used the chemical analysis of bullet lead as a forensic tool for about 40 years. When a bullet(s) is found at a crime scene and in a suspect’s possession the trace elements in the bullet can be compared. If the bullets are similar enough a match is declared. The use of this technique in court testimony was the subject of a relatively recent National Academy of Sciences (NAS/NRC) report. The FBI Crime Laboratory subsequent to the NAS report has ceased using the compositional analysis of bullet lead. We discuss how this procedure died. The issues discussed apply broadly to forensic science laboratory management, chemistry, chemometrics, statistics, and the need for straightforward presentation of scientific evidence.

**Keywords:** Data Analysis, Forensics  
**Application Code:** Homeland Security/Forensics  
**Methodology Code:** Data Analysis and Manipulation
The detection and classification of ignitable liquid residue in forensic fire debris analysis is complicated by interference from pyrolysis products of the substrate materials present in a fire.

A method is developed to derive a set of class-conditional features for the classification of such complex samples. The use of a forensic reference collection allows characterization of the expected variation in complex mixtures of substrate materials and ignitable liquids even when the dominant feature is not specific to an ignitable liquid. Making use of a novel method for data imputation under complex mixing conditions, a distribution is modeled for the expected variation between pairs of samples containing similar ignitable liquid residues. Examining the expected covariance of variables within the different classes allows different weights to be placed on features more important in discerning the presence of a particular ignitable liquid residue.

Performance is evaluated for an ignitable liquid residue classification task using the total ion spectrum (TIS). These measurements include 119 nominal masses measured by GC-MS and averaged across a chromatographic profile. Ignitable liquids are labeled using the American Society for Testing and Materials (ASTM) E 1618 standard class definitions. Classification is performed in the class-conditional feature space wherein new forensic traces are represented based on their likeness to known samples contained in a forensic reference collection. Classification performance using laboratory prepared reference data applied as a model for real burn data achieves 81.4% accuracy. The demonstrated method uses the culmination of information from forensic reference data as the basis of both hard and soft classification assertions.
A sufficient nutrient supply exacerbates unwanted bacterial proliferation in drinking water systems; however locations exist within the treatment process in which bacterial proliferation can be an advantage. Targeting the fraction of labile or easily assimilable organic carbon (AOC) has been applied in the drinking water industry for decades to monitor and assist in approaches to reduce microbial regrowth in distribution systems to maintain optimum water quality. A novel freshwater luminescent AOC test was developed by genetically modifying the traditional test bacteria Pseudomonas fluorescens P-17 (P17) and Spirillum strain NOX with luxCDABE operon fusion and inducible transposons to produce bioluminescent strains. Typically measured through serial dilutions and spread plate counts following extended incubation periods, the AOC assay has been improved by utilizing luminescence as the measurement application. A test for seawater was developed using the marine organism Vibrio harveyi, which is naturally bioluminescent. Both the freshwater and marine AOC test is automated and use a sensitive, photon-counting, luminometer that allows high throughput, replicate analyses, at a low cost. The AOC test is used in drinking water to evaluate biofouling potential and recently in reclaimed water to evaluate regrowth potential in the distribution system. The presentation will discuss a study of elevated AOC levels ranging from 150 to 1,400 µg/liter (n = 146) in reclaimed water that were primarily responsible for bacterial growth and could contribute to the occurrence of opportunistic pathogens such as Legionella and Mycobacterium. Another study of biological filters indicated that 42% of AOC was removed between the influent and effluent even in filters that were incidentally biologically active. The level of AOC provides a monitoring tool for efficient drinking water filtration, observe and control impacts of oxidation and chemical addition, and improve biological stability.

Keywords: Environmental Analysis, Environmental/Water, Luminescence, Method Development
Application Code: Environmental
Methodology Code: Fluorescence/Luminescence
Abstract

Current approaches to determine the presence of emerging (ECs) and other unregulated contaminants in the water bodies are based on discrete measurements. Targeted chemical analysis has been traditionally employed to quantify the concentration contaminants in the environment. The main disadvantage of this approach is to overlook the presence of unidentified chemicals in the water. Combined application of targeted chemical analysis by gas or liquid chromatography tandem mass spectrometry with modern analytical techniques based in advanced mass spectrometry, such as quadruple-time of flight, into environmental monitoring will provide insightful information to environmental specialists to determine the behaviour of ECs throughout water treatment. The current work encompasses the study of the impact of operational conditions during full-scale drinking water treatment on the fate of 8 selected nitrosamines (unregulated disinfection by-products) and their precursors, and 2 selected artificial sweeteners (sucralose and acesulfame K) as indicators of wastewater contamination in the water sources. For this purpose, nitrosamines are analyzed by GC/MS/MS in raw, process and finished water as well in the distribution system from selected American Water (AW) utilities and the sweeteners are quantitated by LC/QTOF in the same locations. Additionally, untargeted ECs are being screened in the source water and samples that undergo in-lab disinfection by the non-targeted analysis capabilities of the LC/QTOF to determine potential transformation pathways involved in the removal/generation of the chemicals under study. In this presentation the results from monitoring nitrosamines and their precursors and other ECs in AW utilities will be shown. The relationship between the concentrations determined in the water and changes in the plant operations and the usefulness of non-targeted analysis to identify unintended consequences of the operational changes will be also discussed.

Keywords: Environmental/Water, Gas Chromatography/Mass Spectrometry, Liquid Chromatography/Mass Spec
Application Code: Environmental
Methodology Code: Other
Using Analytical Solutions to Target Emerging Issues in Drinking Water Safety

Monitoring and Optimizing Drinking Water Treatment Processes with Simultaneous Absorbance and Fluorescence Excitation-Emission Mapping

Conventional optical methods for monitoring drinking water treatment are not generally conducive to process optimization because data collection and processing can be imprecise. Further, chemical analyses are often too slow for effective decision making with many tests taking hours to days to complete while the flow-through the plant is on the order of hours. This presentation outlines the advantages of simultaneous monitoring of absorbance and fluorescence excitation-emission mapping (SAFE) with respect to optimizing control of disinfection by-products, coagulant and granulated activated carbon dosing and detection of algae growth with respect to harmful toxins and taste and odor issues. The SAFE technique collects essential information in seconds to minutes and facilitates immediate processing of several key water quality parameters including: dissolved organic carbon concentration (DOC), an aromatic carbon concentration index superior to conventional specific UV absorbance (SUVA), trihalomethane- and halogenated acetic acid- formation potentials (THMFP and HAAFP) as well as information on microbially and chemically oxidizable substrates that determine the bio- and chemical oxygen demands (BOD and COD, respectively). The method can be applied effectively at the point of the organics laboratory where there is continuous access to key process sampling points including the raw inlet, the settled water and the final plant product. Processing of the data for both qualitative and quantitative purposes can be mediated with automated, calibrated multivariate routines capable of correlating the spectral data with other metrics from online and or batch analysis as well as to recognize and adapt to changes from the normal spectral profiles. In conclusion, the case studies used in the report provide clear evidence that the SAFE technique can provide valuable cost- and time-savings in addition to more precise metering of various drinking water processes.

Keywords: Contamination, Environmental/Water, Fluorescence, Spectrophotometry
Application Code: Other
Methodology Code: Fluorescence/Luminescence
Using Analytical Solutions to Target Emerging Issues in Drinking Water Safety

Emerging Issues Regarding the Impact of Polymer Pipes, Coatings, and Liners on Water Quality

Andrew J. Whelton
Purdue University

Abstract Text
Despite new polymer based materials increasingly entering our water and energy infrastructure systems, there remains little information about how these products (i.e., pipes, coatings, and liners) age and impact the media they contact. As a result infrastructure owners and operators lack the necessary information to make strategic material selection decisions that weigh both product longevity and environmental impacts. Through a series of bench- and field-scale NSF, EPA, VDOT, as well as water and energy industry funded projects we have applied a range of analytical methods for water characterization coupled with material characterization techniques. This presentation will briefly describe the approach and results of these efforts. Gaps in analytical needs and emerging issues will also be discussed. Projects have included the West Virginia Water Crisis scientific investigation, environmental impacts caused by infrastructure rehabilitation technologies, bottled water drinking water safety, green building plastic plumbing systems, biocompatible crude oil spill dispersants, energy generation facility cooling water pipelines, the fate of fracking waste components in water infrastructure, and infrastructure decontamination.

Keywords: Consumer Products, Contamination, Environmental, Polymers & Plastics
Application Code: Environmental
Methodology Code: Gas Chromatography
Using Analytical Solutions to Target Emerging Issues in Drinking Water Safety

Louisiana Response to Detection of Naegleria Fowleri in Two Drinking Water Supplies

During the Summer of 2013 Louisiana experienced the first detection of Naegleria fowleri in a treated drinking water system in the U.S. as a result of an investigation of a related death in 2013 and two previous deaths in 2011. Since this discovery Louisiana has issued an emergency rule requiring a minimum disinfectant residual of 0.5mg/l of free chlorine or 0.5mg/l of total chlorine for systems feeding ammonia throughout the water distribution systems. The emergency rule also requires revised monitoring plans for the Total Coliform Rule as well as increased monitoring for disinfectant residuals. In addition, Louisiana is working to develop lab testing capability in coordination with CDC in our Office of Public Health laboratory in order to perform surveillance monitoring in other public water systems to demonstrate the effectiveness of the increased disinfection levels in controlling the presence of Naegleria fowleri in our public water systems.

Keywords: Environmental/Water, Water

Application Code: Regulatory

Methodology Code: Other
Chemical safety regulations—such as EPA’s Toxic Substances Control Act (TSCA) or OSHA’s Lab Safety Standard 29 CFR 1950—cover a number of regulatory criteria from hazardous materials management to process safety that require research laboratories to have a detailed understanding of the materials being used in the lab. In addition, many of the regulations also have Chemicals of Interest Lists with very specific requirements about managing and reporting those chemicals if and when a certain threshold limit is reached.

Compliance with most of these regulations is extremely challenging. Worse, the frequency with which national, state and local chemical safety regulations are being updated is not only occurring more often, but also increasing in number and complexity. The Globally Harmonized System of Classification and Labelling of Chemicals, or GHS, for instance, that has been adopted by OSHA requires new labels on just about everything in the lab. And right now the details of the upcoming Chemical Safety Act designed to replace TSCA are being argued in Congress; labs will soon need to become thoroughly familiar with the Act’s requirements when it gets signed into law.

Compounding these challenges is the fact that neither regulatory reporting deadlines nor data are coordinated, meaning that if the lab is subject to 10 different regulations there will be 10 different reporting dates and report formats.

This presentation examines how regulations are driving change at research organizations, and how labs are leveraging technology and changing their processes to ensure compliance and reduce chemical risk.

Keywords: Chemical, Environmental, Industrial Hygiene, Laboratory Informatics
Application Code: Industrial Hygiene
Methodology Code: Laboratory Informatics
In 2007, Yale University purchased the property formerly occupied by Bayer Pharmaceuticals in West Haven and Orange, CT. The site is comprised of 20 buildings on 136 acres and includes 1.6 million square feet of research, office and warehouse space. Branded as “West Campus” in 2008, Yale’s ambitious plan is to build a true expansion campus with new and additional programming in basic laboratory research, healthcare, and cultural heritage. To date nearly 1200 faculty, staff, and students call West Campus home and major centers of collaborative academic research have been established on site. Many of these residents are part of West Campus’ six multidisciplinary research institutes, Core laboratories, and the Yale School of Nursing.

Being a true expansion effort Yale is taking advantage of a seldom seen perspective in academics and establishing campus-wide protocols for laboratory operations and compliance at the onset of growth. Additionally, a substantial commitment to capital renovation has led to innovative projects that better connect our multi-disciplinary activities. In this respect the West Campus serves as the ultimate site for evaluating new laboratory support systems prior to full implementation. Yale is also cultivating a culture of laboratory safety by supporting applications, e.g. chemical tracking, through University Administration rather than individual research groups.

This presentation will focus on the successful implementation of campus-wide chemical tracking and its role in furthering the efforts of Yale’s Office of Research Operations and Technology and Office of Environmental Health and Safety. The entire process, from formulation to deployment, will be presented along with logistical lessons learned during the selection of an appropriate software application and implementation team. Similar campus-wide efforts in compliance and innovation will be presented.

Keywords: Chemical, Database, Education, Lab Management
Application Code: Laboratory Management
Methodology Code: Laboratory Informatics
Chemical, Environmental, Industrial Hygiene, Laboratory Informatics

Industrial Hygiene

Laboratory Informatics

Abstract Text
Research organizations that utilize chemicals in their labs and their manufacturing processes must manage those chemicals in a safe environment in accordance with government regulations. At a minimum, to ensure that this is accomplished, a system for managing information about the chemical safety and inventory data should be established and maintained. Best practices, on the other hand, take this minimum and leverage the management of the chemical inventory by taking full advantage of the people, processes, and technology involved. This presentation delves into chemical inventory management best practices that can optimize chemical safety and inventory data, automate audit trails and regulatory reports, manage and mitigate the amount of chemicals on-site, and reduce inventory management costs.
This interesting and entertaining one-hour presentation confronts one of the more common excuses for not having or improving the lab safety program ... “it costs too much.” This is simply not true. Excellent lab safety programs do not need to cost large amounts of money. A few of the 33 simple lab safety program components will be presented and discussed to demonstrate this important theme. These are the critical components for an effective lab safety program.

Participants learn how to create a more effective lab safety program without a purchase order or requisition. Learn how to audit your lab safety program. You don’t want to miss this opportunity for a highly informative, worthwhile and enjoyable learning experience.

Keywords: Chemical, Education, Industrial Hygiene, Laboratory
Application Code: Laboratory Management
Methodology Code: Other
Electronic Laboratory Notebooks (ELNs) are becoming common in both industry and academia. Support for and integration of Analytical Information Markup Language (AnIML) files, used to store analytical instrument data, will become important for ELNs to handle as the framework provides a vendor neutral flat file format, for capturing, annotating and archiving data across a wide range of instruments.

This presentation will discuss the ways in which AnIML could be integrated into an ELN workflow, and the advantages and disadvantages of the approaches.

Keywords: Laboratory Informatics
Application Code: Laboratory Management
Methodology Code: Laboratory Informatics
This presentation highlights the current state of the ASTM AnIML Data Standard. It demonstrates how the standard can be used to deliver analytical data to stakeholders in the organization through web and mobile technologies. We also discuss hosting such applications in the cloud.
Federal Government agencies are working to increase access to the results of research funded by them. This includes both peer reviewed publications and digital data. In order to make this data useful for use by the private and public sectors, the results will need to be made available in standard formats that are widely supported. This presentation will discuss ways in which consensus-based data standards like AnIML can assist in this process.

**Keywords:** Data Mining, Environmental

**Application Code:** Environmental

**Methodology Code:** Laboratory Informatics
Allotrope Foundation is an international consortium of pharmaceutical and biopharmaceutical companies funding a project to develop an innovative Framework (metadata dictionaries, data standards and class libraries) for managing analytical data throughout its lifecycle.

One component of the Framework is a vendor and technique agnostic data standard that defines the structure and format for analytical data and includes rich contextual metadata along with primary instrument data. Another critical and unique aspect of the project is the standardization of contextual metadata (i.e., a core controlled vocabulary which describes key attributes of the experiment and the data) and the mechanism and software tools to utilize that vocabulary in a comprehensive and consistent way throughout analytical workflow. Finally, the Allotrope Framework will provide application programming interfaces (API’s) that will enable software developers to implement the standards in a consistent way. The Allotrope Framework and data format leverages current IT best practices, builds on existing standards, is designed to be extensible, to meet performance requirements of modern instrumentation, and to be resistant to technological obsolescence.

The approach taken by Allotrope Foundation is to learn by doing. The evaluation of any kind of standard (including those for analytical data, workflow context, experimental parameters, services, metadata representation) is done in the context of developing pieces of the Framework and proof-of-concept applications that leverage the standard to solve a particular real world problem. This allows for a deep functional and objective understanding of the degree to which a standard meets the requirements of the Allotrope Framework.

The presentation will describe the understanding gained in evaluations of AnIML and other standards, the Framework development to date and discuss plans for the adopted standards and future extensions of the Framework.

Keywords: Data Mining, Informatics, Scientific Data Management, Software
Application Code: Pharmaceutical
Methodology Code: Laboratory Informatics
The SiLA Consortium aims to establish global standards to make laboratory automation more efficient and profitable. SiLA is a non-profit, international consortium and joins companies, research institutions and individuals in a global community. Among our SiLA members working together in various working groups are leading device manufacturers, software companies, system integrators and Pharma/Biotech enterprises as well as lab automation experts from all over the world.

What is the basic idea of SiLA?
SiLA defines standardized interfaces for device control and communication such that users can easily integrate and rearrange instruments following a "plug and play" philosophy. By using a SiLA interface, there is:
- less effort in integrating devices from different vendors
- more flexibility to reuse lab equipment in various setups
- efficient and cost-effective use of laboratory automation systems over the entire lifecycle

With increased lab efficiency, scientists are able to run more experiments in shorter time frames, generating larger amounts of data than ever before. SiLA goes beyond just standardizing device communication interfaces and extends its standardization philosophy to data handling through its partnership with the “best in class” data handling solution, AnIML.

“AnIML via SiLA” uses SiLA as the standardized “Transport Vehicle” for “AnIML Containers” of data. The combination of both standards results in an even more complete solution to be applied in labs right now!

SiLA is quite excited about the past years of progress and is gaining momentum! There are already many vendors that supply systems compliant to the SiLA Device Control and Interface Standard. Making them “speak” AnIML would be a major leap forward for both standards and most of all for users and scientists in the labs!

In this workshop, we would like to provide some more insights on SiLA and discuss real world applications of “AnIML via SiLA”. We hope to see you there.

Abstract Text
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Keywords: Lab Management, Laboratory Automation
Application Code: Laboratory Management
Methodology Code: Laboratory Informatics
Analysis of trace level of ions in the presence of high levels of matrix ions is challenging.

In recent years, a new ion chromatography analysis methodology termed two-dimensional Matrix Elimination Ion Chromatography (MEIC) has been introduced to meet this specific challenge. MEIC takes advantage of large capacity columns and stationary phase selectivity to minimize the interference from the common ions in the first dimension. The analytes of interest are refocused in a concentrator column and reanalyzed in the second dimension using a smaller format column with a different separation phase to achieve better sensitivity and selectivity in the second dimension. The second dimension column can thus be a microbore format column or a capillary column.

In this presentation, we will show the application of MEIC for the analysis of various trace contaminants in drinking waters. Overall the method is automated and eliminates the tedious manual processes currently employed and provides improved analysis from a sensitivity, precision and recovery perspective.

Keywords: Chromatography, Environmental Analysis, Environmental/Water
Application Code: Environmental
Methodology Code: Liquid Chromatography
On February 11, 2011, the United States Environmental Protection Agency (USEPA) initiated development of a national primary drinking water regulation (NPDWR) proposal for perchlorate. There are several published methods for the analytical determination of perchlorate in raw and finished drinking waters. The robustness of these methods is limited by interferences, false positives, ionization suppression, and detector inlet or column fouling. This work discusses a single laboratory validation of EPA Method 314.2. Calibration model, precision, accuracy, single laboratory lowest concentration minimum report (LCMRL) limit in DI water and detection limit were evaluated. The calibration model was determined by analysis of six calibration levels over six days. Residual values were examined to determine if weighting was necessary. ANOVA calculations were performed to determine the appropriate order of calibration model. A lack of fit test was performed as a final evaluation of the calibration model. Precision was determined by examination of relative standard deviation for eighteen replicates at three levels in both DI water and synthetic matrix. Accuracy was determined by analyzing samples spiked with the analyte. Additional considerations are given to analysis of matrices containing high ionic strength and a comparison between first dimension and second dimension data.

Keywords: Chromatography, Environmental/Water, Ion Chromatography, Water Application Code: Environmental Methodology Code: Gas Chromatography/Mass Spectrometry
Aquariums are an important part of modern society, as they provide both a way to explore the wonders of the aquatic world and a source of entertainment. Assaying aquarium water is essential for the proper maintenance of aquariums in order to provide fish and other sea creatures with the healthy environment they need to thrive. These water samples offer a significant analytical challenge in that they often contain a wide variety of dissolved salts and/or organic molecules at greatly differing concentrations. Common anions/cations are found at very high concentrations, and contribute significantly to the overall salinity of sea water. Other ions, such as strontium (an essential element used by reef-building invertebrates) or iodide (also crucial to a variety of marine life) are found at much lower, or even trace, levels. Ion profiling of artificial seawater using ion chromatography followed by suppressed conductivity detection was investigated as a means to ensuring proper tank condition. Maintaining stable water chemistry of aquarium systems requires frequent sampling and, as a consequence, high throughput is a desired analytical characteristic. The goal of the project was to improve, optimize, and validate a currently-in-place method for the determination of cations and anions in artificial seawater. Variables such as mobile phase composition, column/cell temperature, and injection loop volume were studied in order to achieve better peak resolution in a shorter time period. The impact of a validated ion profiling method on aquarium maintenance is an important contribution to the zoo and aquarium industry.

Keywords: Analysis, Detection, Environmental/Water, Ion Chromatography
Application Code: Environmental
Methodology Code: Liquid Chromatography
New Developments in Ion Chromatography

**Fronting, Tailing and Non-Gaussian Peaks: How Can We Predict the Peak Shapes in Ion Chromatography?**

Ion chromatography (IC) is one of the most popular techniques for the separation and analysis of inorganic and small organic ions. Normally, chromatographers expect perfectly Gaussian peak shapes in liquid chromatography. With the introduction of high efficiency phases and small particle sizes excellent peak shapes with high efficiency phases are commonly seen. However, many challenging and real samples contain disproportionate amounts of analytes in the sample. Such samples can cause mass overload. In such cases, non-Gaussian peak shapes are seen. These fronting and tailing peaks shift the retention times of minor components and are therefore important in two dimensional liquid chromatography.

Our efforts are directed at understanding the fundamental basis of peak shapes with special reference to ionic analytes. This talk will discuss simple practical and predictive rules for determining the peak shape of a given ion on a modern high efficiency IC column. These simple ideas will allow chromatographers to predict the peak shapes without knowing the actual column chemistry and without resorting to complex numerical analysis. Finally, we will present two interesting cases (1) "Is it possible to see both fronting and tailing on the same IC column using the same mobile phase?" and (2) Can fronting and tailing peaks shift the retention times of other components under mass overload?

**Keywords:** Analysis, Ion Chromatography, Ion Exchange, Trace Analysis

**Application Code:** General Interest

**Methodology Code:** Liquid Chromatography
Although ion chromatography is a relatively mature science, research into the development of new stationary phases for ion chromatography continues at an active pace more than 35 years after the introduction of ion chromatography as an analytical technique. In this work I will review the latest developments in new ion exchange phases developed specifically for ion chromatography. We will cover a variety of different stationary phase architectures and include examples illustrating their application to water quality and environmental analytical challenges.
We describe an admittance detector for high impedance systems (small capillary bore and/or low solution specific conductance). Operation in the low frequency range (<1 kHz, much lower than used heretofore) provides optimum response to conductance changes in capillary id <20. The detector design was based on theoretical studies. The highest S/N for detecting 100 μM KCl injected into water carrier (5.5 μM peak concentration, ~0.8 μS/cm) in a 15 μm i.d. capillary was observed at 500-750 Hz. A low bias current operational amplifier in the transimpedance configuration permitted high gain (1 V/nA) to measure pA-nA level currents in the detection cell. Aside from an oscillator, only one more chip, a RMS-DC converter that can also provide offset, formed the complete detection circuitry; an additional operational amplifier can provide for added gain and further offset capabilities. Limits of detection (LODs) of KCl scaled inversely with the capillary cross section and were 2.1 and 0.32 μM injected KCl for 2 and 5 μm id capillaries, respectively. Used as a detector on a 16 μm id polymethylmethacrylate capillary in a split effluent stream from a suppressed ion chromatograph, the LOD was 27 nM bromide (Vex 22 V p-p), compared to 14 nM observed with a commercial bipolar pulse macroscale conductivity detector with an actively thermostated cell. We also show applications of the detector in capillary electrophoresis in the smaller capillaries. Efficient heat dissipation permits high concentrations of the background electrolyte and sensitive detection because of efficient electrostacking.

**Keywords:** Capillary Electrophoresis, Capillary Ion Analysis, Instrumentation, Ion Chromatography

**Application Code:** Environmental

**Methodology Code:** Separation Sciences
The utility of a suppressor in Ion Chromatography is well established. From the introduction of the packed bed suppressor to the continuously regenerated suppressors there has been significant progress in this arena. The development of a continuously regenerated suppressor paved the way to a more robust analytical platform for pursuing ion analysis. Advances in regeneration such as by electrolysis resulted in a more easy to use suppressor system. The recycle mode made it even more convenient and for the first time ion analysis was feasible with just an eluent containing reservoir. Advances in the eluent generation technology resulted in a system that today could be operated purely with a deionized water stream.

In the first part of this presentation we review the suppressor technology from a historic perspective. In the second part of this presentation we discuss a newly introduced electrolytic suppressor called the Electrolytically Regenerated Suppressor (ERS 500). We will discuss the design details along with the key benefits. Other designs of the suppressor particularly geared towards improving the chromatographic performance will be discussed. A new chemical suppressor design will also be discussed. We will also show example performance characteristics of the new suppressors. Results from analyzing real life samples such as drinking and waste waters will also be shown.

Keywords: Environmental Analysis, Ion Chromatography, Ion Exchange, Water
Application Code: Environmental
Methodology Code: Other
An Overview of Supercritical Fluid Chromatography Mass Spectrometry (SFC-MS) in the Pharmaceutical Industry

Although SFC-MS has been around since the late 1980s, it has not become as prevalent as LC-MS in the pharmaceutical industry because, until recently, most of the work was in the form of preparation SFC chromatography for isolating compounds (usually chiral). However, with more sensitive instruments coming online, the analytical interest in SFC has reawakened and along with that has come an increase in SFC-MS activities. This review follows the progression of SFC-MS technology, the types of interfaces, back pressure regulators and analyzers that have been coupled to SFC. An overview of chiral SFC-MS and prep SFC-MS in the pharmaceutical industry is presented.
In recent years, core shell particles have gained interest for use in HPLC and SFC applications. The hallmark of core shell particles are their relatively rapid mass transfer properties, making them ideal for high speed analytical separations. Good mechanical stability (strength) of the particles also lends itself to these fast and often high pressure separations in HPLC. While core shell materials have received a lot of attention for UHPLC application, less work has been done in the SFC realm. This is mainly due to the lack of variety of stationary phases available on core shell supports and the inherent “speed” already built in to SFC separations with traditional porous silica based stationary phases. However, as our industry trends to smaller particles and faster analysis times, even SFC users can benefit from core shell materials. We will present comparative data on three SFC stationary phases, bonded both to a core shell support and an analogous porous silica support. We will examine chromatographic similarities and differences over a wide range of commercially applicable test compounds. The data will illustrate in many cases that porous silica stationary phases, if properly optimized for SFC, can challenge core shell separations with respect to speed and selectivity.

**Keywords:** Chromatography, SFC, Supercritical Fluid Chromatography

**Application Code:** Pharmaceutical

**Methodology Code:** Supercritical Fluid Chromatography
Process analytical chemistry serves as a critical function in the drug development process. A variety of analytical techniques are needed to support the analytical research and development activities. Supercritical Fluid Chromatography (SFC) has been used in process analytical chemistry to meet the increasing demand for better efficiency. In this presentation, the typical applications of Supercritical Fluid Chromatograph (SFC) in process analytical chemistry will be discussed with highlighted examples, especially in the area of chiral separations.
The aggressive pace of pharmaceutical drug discovery programs has placed increasing emphasis on implementing innovative technologies for processing new compounds in a rapid and efficient manner. The chromatographic efficiency of supercritical fluid chromatography (SFC) has enabled our group to rapidly characterize and isolate lead candidates for screening and development since early 2012. A mass-directed preparative SFC system is suitable for fast compound separations and same-day recovery of both chiral and achiral compounds at high specificity and sensitivity. The orthogonality of SFC enables successful separations of complex mixtures or chiral isomers that often limit the use of traditional techniques such as normal or reversed phase chromatography. Timely chiral purity checks for multiple projects have ensured a compounds’ purity and identity to alleviate toxicity concerns.

This presentation will cover the implementation of SFC, instrument design optimizations, and strategies for successful small molecule separations in an automated high-throughput laboratory setting. Examples highlighting the benefits of SFC in pharmaceutical achiral-chiral separations and analysis will be discussed, especially for solving critical program challenges in large scale purifications and chiral separations of drugs and related metabolites.
### Chiral Method Development in SFC with Laser Polarimeter Detection

Overview of chiral method development techniques in SFC with laser polarimeter detection. Discusses screening techniques, methods, columns, solvents, elution order, solubility, etc.

**Keywords:** Chiral Separations, Method Development, SFC, Supercritical Fluid Chromatography

**Application Code:** Drug Discovery

**Methodology Code:** Supercritical Fluid Chromatography
Chiral Chromatography has become essential for the pharmaceutical industry since there is a new focus on homochiral drugs. There are three different modes of chromatography that are utilized for nearly all of these chiral applications. Each mode: Super Critical Fluid Chromatography (SFC), Normal Phase Chromatography (NP), and Reverse Phase Chromatography (RP), has its own strengths and weaknesses. Since enantiomers are mirror images of each other and practically all of their physical properties are exactly the same. The development and execution of the chromatographic separations of chiral compounds are very different than for any other chromatographic methodology. It is virtually impossible to predict chiral resolution for a particular pair of enantiomers without some prior knowledge.

Chiral chromatographic method development always starts with screening the chiral compounds on columns capable of chiral separations. This is where the differences between the modes of chromatography become important. SFC is capable of screening compounds and columns much faster than NP or RP because the mobile phase can run at high linear velocities due to the eluents low viscosity and the columns equilibrate rapidly. However, the equipment used for SFC has the install base out of these three modes in the pharmaceutical industry. RP is easily the most common mode of chromatography used in the industry and NP uses the same equipment as RP but sometimes needs small changes due to solvent compatibility. There are reasons why someone might want chiral methodology for the same compound on several chromatographic modes. It might have to do with equipment availability, their regulatory environment, or in what solvent their samples solutions arrive in. The work described here will use real examples to demonstrate how chiral methodology can be screened in one mode and switched to another mode of chromatography.

Keywords: Chiral, Method Development, Pharmaceutical, Supercritical Fluid Chromatography
Application Code: Pharmaceutical
Methodology Code: Supercritical Fluid Chromatography
On occasion an analytical chromatogram presages a preparative separation. The purpose of the preparative separation may be to purify the main component from impurities found in the analytical chromatogram, to isolate enantiomers from a racemate or to isolate small amounts of impurities of impurities for identification. The equipment and procedures involved greatly depend not only on the object but also on the scale of the separation.

Scale-up from the analytical separation to preparative use is straightforward providing some simple rules are followed. This may involve not only a change in column size but also a change in packing material – both its dimensions and perhaps its nature. Guidelines in making this transition – when it is appropriate to redevelop a method and when to use one as close as possible to the existing one – are given. The isolation of impurities should not be done in a single stage. A pre-purification step is essential to make the economics of the project viable. One approach is the supercritical fluid extraction of the impurities from the product; an illustration using extraction of a sample as an injection procedure to concentrate and make an initial purification of the sample is presented.

The question of the appropriate size of the preparative equipment can be approached by analysis of the costs of the operation. If one takes into account the operating costs, the costs of manpower, facilities and services as well as the cost of amortization of the equipment it is clear that there is an optimum size system for any specific project size. Data are presented demonstrating that where a single system is to be used to cover all scales of separation in a laboratory, it is usually best to choose a unit appropriate for the larger scale separations; the costs of running a small separation on a large system, although not optimum, are in general less than those of running a large separation on a small one.

Keywords: Chiral Separations, Pharmaceutical, Prep Chromatography, Supercritical Fluid Chromatography
Application Code: Pharmaceutical
Methodology Code: Supercritical Fluid Chromatography
Human metabolites are a carrier of comprehensive information on the status of the entire metabolism with regard to nutrition, diseases and progress of a therapy, medication, drug abuse and nutrition. However, in general sampling, enrichment and adsorption steps are required before transport to a laboratory for analysis. Therefore, results are available only with a delay and quantification is limited. We want to present a method using ion mobility spectrometry coupled to rapid gas-chromatographic pre-separation and combined with appropriate sampling which enables the on-site, non-invasive sampling and analysis of the volatile metabolites in exhaled breath or sweat. When the focus is on semi-volatile or non-volatile metabolites this could be realised with the same analytical technique but coupled to e.g. laser desorption or electro spray ionisation. As a data base with the relevant compounds and suitable software algorithms for data evaluation are available, the results of such an analysis are available after few minutes and can be used e.g. for diagnosis.

Keywords: Clinical Chemistry, Forensics, Medical, Metabolomics
Application Code: Clinical/Toxicology
Methodology Code: Chemical Methods
Dispersion that occurs during flow injection (FI) was investigated earlier by our research group to detect and correct for matrix interference in inductively coupled plasma time-of-flight mass spectrometry. The concept is straightforward: in the absence of an interferent, the dilution that occurs during FI should be the same for all sample constituents. As a result, the ratio of any two elemental signals should be constant. However, when an interferent is present, the signals from individual elements are affected differently, so the signals deviate from a constant value, especially at the peak of the FI curve where the interferent concentration is greatest. The drawback of this earlier method was that dispersion, and therefore dilution, was element-specific, causing the ratios to wander even when no interference existed. Here, we employ a gradient HPLC pump to overcome this drawback. With longer gradient runs and smaller gradient steps when compared to flow injection, the dispersion variation between elements is negligible and difficulties associated with it are eliminated. Importantly, when an interference exists, the optimal dilution factor to reduce the interference to an acceptable level can be found from the gradient curve as the point where the signal ratio between two elements becomes constant.

Abstract Text
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Keywords: Atomic Spectroscopy, Flow Injection Analysis, ICP, Mass Spectrometry
Application Code: Quality/QA/QC
Methodology Code: Chemical Methods
### Session Title
Chemical Methods and High-Throughput Chemical Analysis

### Abstract Title
High-Throughput Microplate-Based Microcolumn Device for the Selection of Aptamers, Characterization of the Selection Process, and Other Chromatographic Processes

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### Abstract Text

We present a high-throughput device designed for large-scale aptamer selections, characterization and optimization of the selection process. This versatile device is also capable of performing chromatographic processes, including group exclusions and charge-based separations. This device, Microplate-based Enrichment Device Used for the Selection of Aptamers (MEDUSA), is an extension of our previously developed microcolumn technology and comprises 96 microcolumns arranged with the dimensions of a 96-well microplate, allowing it to be coupled directly to a microplate. It is a layered device fabricated using CO\(_2\) laser machining, which enables it to be customized or reconfigured, with the ability to be assembled such that the microcolumns are connected in series or in parallel. MEDUSA has been used to characterize aspects of the aptamer selection process, including the specificity and partitioning efficiency of specific aptamers. A smaller customized version of MEDUSA was used to perform RNA aptamer selections to 12 protein targets with a single aliquot of nucleic acid library using the columns connected in series for the initial binding step, and in parallel for the subsequent steps. The results of these experiments, as well as chromatographic separations using the microcolumns will be discussed. MEDUSA’s plate-based format allows easy integration with other plate-based systems for downstream biochemical processes and analysis.

This work was supported by the National Institutes of Health, and was performed in part at the Cornell Nanoscale Facility, a member of the National Nanotechnology Infrastructure Network, and supported by the National Science Foundation.

### Keywords
High Throughput Chemical Analysis, Liquid Chromatography, Sample Handling/Automation, Sample Preparations

### Application Code
Bioanalytical

### Methodology Code
Chemical Methods
Multistep assays are often performed by using either magnetic beads or filter plates with a vacuum manifold. Magnetic beads are a popular tool for such assays, however they suffer from several disadvantages including the aggregation of the magnetic beads, the need for powerful magnets and the possibility of bead loss during washing steps. Vacuum based systems suffer from difficulties regulating vacuum pressure well-to-well and plate-to-plate. In addition, they have difficulty adapting to solvents of various polarities and viscosities.

We have devised a method for optically gating the passage of fluids through microtiter plate filter floors by functionalizing the floors with photochromic spiropyran derivatives. Spiropyrans are a class of organic compounds known for their photochromic properties; they are reversibly isomerized between a closed, hydrophobic form and an open hydrophilic form upon irradiation with UV and visible light, respectively. Photo-controlled gating of the filter floor allows for more rapid, efficient assays and a reduction in sample loss by enabling automated reagent transfer out of the wells between treatment steps.

The bottom surfaces of the wells are functionalized by covalently bonding spiropyrans to the membranes. Photogating is accomplished by irradiating the floor of the microtiter plate with the appropriate wavelength of light to open or close the spiropyran, rendering the floor hydrophilic (enabling passage of aqueous solutions) or hydrophobic (effectively “closing” the filter floor to passage of aqueous solutions). This presentation provides an overview of methods and applications of the innovation, along with surface characterization data.
In recent years, laboratory managers have faced challenges in procurement and management of funds due to decreasing budgets, and as a result, there is an urgent and ongoing need to improve laboratory efficiency. Instrument manufacturers are challenged to provide researchers with higher-throughput analytical methods that do not compromise the quality of results but still meet budget requirements. An automated sampler handling system (EasyPREP) was acquired to reduce labor costs and to improve precision and accuracy, thereby increasing laboratory efficiency. The EasyPREP system allows for automated preparation of calibration standards, addition of multiple reagents, and internal standard additions and is suitable for many solvents. The instrument was procured to perform routine extractions of PM$_{2.5}$ air samples collected on Nylon and Teflon filters. Prior to acquiring the EasyPREP system, PM$_{2.5}$ samples were extracted using manual air displacement pipettes.

Samples were extracted using both the manual and automated delivery methods, and results were evaluated for precision and accuracy. The precision for samples extracted using the EasyPREP automated system was nearly 40 times better than the precision calculated for samples extracted using manual pipettes. Accuracy of the EasyPREP automated system was within 0.5% for 25.0 mL volume deliveries and 0.3% for 20.0 mL volume deliveries. Precision and accuracy were evaluated in a year-long study to determine consistency of the automated system and found to be reliable and steady throughout the year.

In conclusion, the EasyPREP automated system has been demonstrated to be a more reliable and effective method than manual pipetting. It reduced labor costs, increased efficiency and throughput, and maintained high precision and accuracy.

Funding for this research was provided by Research Triangle Institute.

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Funding for this research was provided by Research Triangle Institute.
Chemical Methods and High-Throughput Chemical Analysis

Extending Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) to Detection of Semi-Volatiles on Surfaces

Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) (Smith & Spanel, 2005) is a real-time analytical technique that detects volatile organic compounds (VOCs) and certain inorganic gases to low part-per-trillion concentrations (by volume; pptv) without sample preparation or preconcentration (Prince et al., 2010). These characteristics mean that SIFT-MS has traditionally been applied to real-time detection of VOCs in air.

This paper presents the very recent development of a swab desorber inlet that has for the first time enabled SIFT-MS to be applied to detection of diverse semi-volatile organic compounds (SVOCs) swabbed from surfaces. Very low nanogram quantities of SVOCs are readily detectable from a single swab, with greater selectivity than ion mobility spectrometry (IMS) due to the use of three instantaneously switchable reagent ions (H$_3$O$^+$, NO$^+$ and O$_2$$^+$) coupled with mass spectrometric detection.

Compounds detected using the swab-based SIFT-MS analysis include explosives, explosives taggants, drugs of abuse and their precursors, and pungent odor compounds. A detailed presentation will be made on detection of taint compounds (such as dichlorophenol and tribromoanisole) that permeate through packaging materials and taint food and pharmaceutical products. This example also illustrates how readily SIFT-MS can be applied as a turn-key application.

Acknowledgement: This work was funded by Syft Technologies Ltd, New Zealand.


Keywords: Mass Spectrometry, Semi-Volatiles, Thermal Desorption, Trace Analysis
Application Code: High-Throughput Chemical Analysis
Methodology Code: Mass Spectrometry
Chemical Methods and High-Throughput Chemical Analysis

Reducing Helium Use in Flowing Atmospheric-Pressure Afterglow—Mass Spectrometry

Many plasma-based sources for ambient desorption-ionization mass spectrometry (ADI—MS) perform best with helium as a supporting gas. The flowing atmospheric-pressure afterglow (FAPA) source is one such example, and uses around 1.0 L He/min. Unfortunately, even this modest flow rate can be cost-prohibitive, especially in some countries, and is best to avoid everywhere, since helium is a non-renewable resource. To address this problem, we have explored several strategies to reduce helium consumption; one successful strategy includes pulsing the gas flow on for only short periods and just when samples are immediately present in front of the source. Due to residual He in the discharge cell, the plasma can be sustained between He pulses with no gas flow for several minutes. When the helium flow is then restarted, the plasma quickly returns to its original state for a selected analysis period. Accordingly, helium is conserved as a function of the effective duty factor of gas-flow pulses. Gas flow, current-voltage characteristics, and helium pulse rate and duration have been studied and optimized. Sensitivity, repeatability of measurement, plasma behavior, and mass-spectral characteristics of this system will be discussed and advantages and drawbacks of pulsing the helium flow will be considered.

Keywords: Chemical Ionization MS, High Throughput Chemical Analysis, Mass Spectrometry, Plasma

Application Code: High-Throughput Chemical Analysis

Methodology Code: Mass Spectrometry
Buffer capacity \( (B) \) is a superior parameter to pH because it is an estimation of the stability of pH at a particular time as opposed to just a static reading of the hydrogen ion activity. Physiologically, a blood pH of 7.4 is considered normal but there are no indications of how stable that pH is or how fast it will change. Buffer capacity is simply defined as the total acid or base influx a system can sustain without causing a pH change. The body's overall capacity is accounted for through contributions predominantly from the carbonic acid – bicarbonate buffer system, phosphates and plasma proteins. Acid is one of the major by-products of normal physiological activity and the blood's total capacity protects the body against this acid influx and aids in the removal of these toxins. Patients with sickle cell disease (SCD) and traumatic injuries have compromised homeostatic adaptations that cause an acid build-up and eventually reduce tissue pH. From the point of view of diagnostics, no point-of-care technologies exist that quantify the body's overall ionic composition reliably and the systems that do present an estimation of the blood’s overall ionic composition are multi-parametric, expensive, time-consuming and not portable. This project focuses on engineering a lab-on-chip device to quantify the buffer capacity of small whole blood samples (<1 ml) ex-vivo whilst addressing key challenges of preserving sample integrity by preventing clots and atmospheric gas effects. A unique coulometric titration device was developed to generate a constant proton flux required to systematically titrate all buffering sources in whole blood. A comparative understanding of the time taken to reach the buffer’s titration end point generates an understanding of the titration curve and an estimation of the total buffer capacity in a matter of minutes. The device is intended to aid clinicians attending to patients with traumatic injuries to effectively reduce multi-organ failure rates and also help clinicians prevent vaso-ocular episodes in patients suffering from SCD.
Electrochemical Microfluidics for Bio-Analysis
Cancer Biomarker microRNAs Detection Based on Graphene by Electrochemical Method in Cell Lysates

An electrochemical nucleic acid biosensors for the purpose of detection of microRNAs from cell lysates will be discussed. The fabrication of the system has been done via immobilization of complementary antimir-microRNA to graphene (GRP) modified pencil graphite (PGEs) electrodes prior to solid phase hybridization with either synthetic or microRNA contained total RNA. Enhancement of the signal due to GRP and the existence of GRP on PGEs have been investigated via electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV). Intrinsic guanine oxidation signal recorded via DPV and charge transfer resistance, Rct values determined via electrochemical circle fit option of EIS. Designed biosensor with its LOD of 1 pmole is applicable to analysis of certain miRNAs from total RNAs isolated from cell lysates. Many studies have been done in our lab related to microRNAs [1-4].

GRP modification of bare PGE; optimized amount of GRP has been added to the acetate buffer solution (ABS) and sonicated for 20 minutes at room temperature. The bare PGEs are activated in the ABS (with 20 mM NaCl, pH:4.8) by applying +1.40 V for 30 s. A novel, ease-of-use, rapid, reproducible and selective graphene based electrochemical microRNA biosensor has been developed. To optimize hybridization conditions, parameters affecting the process of immobilization and hybridization time, buffer pH, concentration of probe and target sequences have been studied.


Keywords: Biomedical, Biosensors, Biotechnology, Voltammetry
Application Code: Biomedical
Methodology Code: Sensors
Abstract Text

The increasing prevalence of antibiotic resistance challenges researchers to better understand infection, propagation of disease, and innate immunity. Group B streptococcus (GBS; S. agalactiae) is a beta-hemolytic Gram-positive bacterium that asymptomatically colonizes approximately 20-30% of healthy women; however, it can cause severe disease in elderly, immunocompromised adults and neonates resulting in pneumonia, septicemia, and meningitis. Although GBS can be screened for and treated prior to delivery, GBS infections remain a leading cause of chorioamnionitis, preterm birth, stillbirth, and neonatal sepsis throughout the developing world. To understand the metabolic differences between a hypervirulent neonatal invasive strain (GB37) and an asymptomatic maternal vaginal colonizing strain (GB590), the multianalyte microphysiometer (MAMP) was utilized to dynamically measure glucose and oxygen consumption, lactate production, and extracellular acidification simultaneously. In addition to monitoring the differences between invasive and colonizing strains, further studies were performed to elucidate the effect of prostaglandin E2 (PGE2), an immunomodulatory lipid molecule found in high concentrations in the pregnant uterus and thought to mediate inflammatory responses throughout gestation. To simulate the macrophage-rich placenta, a murine macrophage-like cell line, RAW 264.7, was exposed to both strains of GBS, with and without the presence of PGE2. These studies provide real-time measurements of GBS exposure to macrophages and highlight the differences between strain virulence, enhancing the understanding of GBS infection and proliferation, which may eventually lead to accessible and early diagnostic tools for future screening.
Octopamine is an endogenous biogenic amine that plays important roles as neurotransmitter, neurohormone, and neuromodulator in invertebrates, and has functional analogy with norepinephrine in vertebrates. In the past, fast scan cyclic voltammetry (FSCV) has been used for real time detection and characterization of rapidly changing dopamine and serotonin in the [i]Drosophila melanogaster[/i] larva ventral nerve cord (VNC). Release of neurotransmitters has been controlled by optogenetic activation of blue light sensitive channel, Channelrhodopsin (ChR2). Blue light causes a false peak near the upper (switching) potential of the FSCV waveform, which is close to where the octopamine oxidation peak is observed. This non-faradaic false peak could be due to tissue damage or ionic changes, and makes it difficult to detect blue light mediated octopamine release. We have characterized evoked octopamine release with two new stimulation methods, the ATP sensitive channel, P2X2, and a newly discovered channelrhodopsin, CsChrimson, which is red light sensitive. On average, a 5.0s stimulation with CsChrimson evokes 0.11µM of octopamine release in the larval VNC. Current due to evoked octopamine decreases upon repeated stimulation, and varying the inter-stimulation time does not change the magnitude of current decrease. The release is reduced by reserpine, demonstrating that it is vesicular. Overall, the ability to stimulate and detect octopamine release will allow a more thorough characterization of this important neurotransmitter in [i]Drosophila[/i].
In recent years there has been considerable interest in the development of continuous physiological monitoring technologies for assessing physical performance in athletes. Measurement of metabolites is of particular interest as it provides information about the metabolic state of the tissue.

We are developing a wearable microfluidic analysis system that uses biosensors to quantify key metabolic markers of exercise. Microdialysis is used to sample the subcutaneous tissue during various levels of cycling and during the post-exercise recovery period, and the resulting dialysate is monitored for changes in glucose and lactate levels. The system consists of microelectrode-based amperometric biosensors [1] within a 3D-printed microfluidic flow-cell. Using this system we are able to continuously monitor the metabolic state of subcutaneous tissue in real-time.

Results will be presented from cyclists during and after various levels of exercise. These will be compared to results obtained using our existing human rsMD system [2,3].

References:

Keywords: Bioanalytical, Biosensors, Electrochemistry, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Electrochemical Microfluidics for Bio-Analysis

Redox Chemistry of Nanoceria Using Impedance Spectroscopy

Cerium oxide nanoparticles, also known as nanoceria, have been proposed as potential radical scavenger, an abrasive catalyst in chemical polishing, a catalyst in fuel cell and as therapeutic molecules. The autocatalytic and therapeutic properties of nanoceria are derived from its redox chemistry. Ceria exists in both $+3$ and $+4$ states due to oxygen vacancies created during their formation. The redox value is sensitive to pH; however, its redox chemistry is not readily assessed using conventional electrochemical techniques such as cyclic voltammetry or chronoamperometry. This paper presents the ac electrochemical impedance spectroscopy (EIS) characterization of nanoceria. The effect of the maximum phase angle ($\phi_{\text{max}}$) the interfacial charge transfer resistance ($R_{\text{ct}}$) and capacitive charge transfer ($C_{\text{dl}}$) at varying concentrations, sizes and pH will be presented. The impact of these parameters on the catalytic properties of nanoceria will be discussed.

Keywords: Detection, Electrochemistry

Application Code: Bioanalytical

Methodology Code: Electrochemistry
Our understanding of the underlying mechanisms of action of anesthetic drugs, and their influence on neural transmission, is relatively limited. Understanding how anesthetics affect neuronal activity would be advantageous in studies exploring brain circuits. Here, we investigated the influence of isoflurane or urethane on basal dopamine levels within several brain regions including the nucleus accumbens (NAc), the anterior cingulate cortex (ACC), and the caudate putamen (CPu) using fast-scan controlled adsorption voltammetry (FSCAV). Fast-scan cyclic voltammetry (FSCV) was used to measure dopamine release with millisecond resolution in the NAc following electrical stimulation in the medial forebrain bundle. Evoked release was determined in the absence or presence of cocaine (20 mg/kg, i.p.). Urethane was given intraperitoneally at 1-1.5 gm/kg and isoflurane was constantly administered at 1.25%; level of anesthesia was monitored in individual rats by monitoring respiration rate and by presence of reflex response to nociceptive stimuli. Basal dopamine concentrations were lower in the ACC, than in the CPu and NAc in isoflurane anesthetized rats but similar across regions in urethane anesthetized rats. In the presence of cocaine, tonic [DA] was increased similarly in all anesthetized animals but showed an extended duration in urethane over isoflurane, anesthetized rats. Additionally, electrical stimulation in the presence of cocaine produced a greater increase in NAc phasic dopamine release in urethane, than in isoflurane, anesthetized rats (135 and 84% over baseline). These findings indicate differential effects of anesthesia on dopamine dynamics and provide a basis for standardizing conditions of anesthesia in experimental settings.
We present an origami paper-based electrophoretic device (oPEpD), which achieves rapid (< 5 min) separation of fluorescent molecules and proteins at a voltage of 10 V, more than 10 times lower than that in a regular electrophoresis apparatus. oPEpDs use multilayer origami paper as supporting medium for electrophoresis, which significantly shortens the distance between anode and cathode (e.g. ~2 mm thick for an 11-layer origami paper) and thus, generates a strong electric field > 1 kV/m even with a few volts input. In addition, the multilayer structure of oPEpD enables convenient sample introduction by a slip layer, as well as easy product reclamation and analysis after electrophoresis by unfolding the device and cutting out desired layers. These advantages—low cost, low voltage, speed, and ease of use—make the oPEpD a potential platform for point-of-care (POC) analysis. The use of oPEpDs for simple separation of proteins in bovine serum is also demonstrated, which indicates its potential applications in diagnostic testing.

**Keywords:** Bioanalytical, Electrophoresis, Lab-on-a-Chip/Microfluidics, Portable Instruments

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Preterm birth (PTB) is the leading cause of infant death. Although the etiology is unknown, 6 protein and 3 peptide serum biomarkers were identified in association with spontaneous PTB. Even though no single biomarker had the specificity and sensitivity to diagnose PTB, a panel of these 9 has the predictive power to greatly aid clinicians in diagnosing PTB risk. We are focusing on creating a timely, accurate, inexpensive, miniaturized, and automated device that can selectively concentrate, label, separate, and quantify PTB biomarkers. Our approach entails a microfluidic lab on a chip that combines selective extraction on an affinity monolith column, labeling with a fluorescent species on a reverse-phase monolith column, electrophoretic separation, and quantitation by laser-induced fluorescence detection. We are currently optimizing conditions for on-chip labelling, elution of the unreacted dye and electrophoretic separation of the biomarkers. These devices should allow diagnoses of PTB risk prior to contractions and membrane rupture, which increases intervention and treatment options.

Funding was provided by the National Institutes of Health (R01 EB006124) and an Undergraduate Research Award from the Department of Chemistry and Biochemistry at Brigham Young University.

References
Microfluidic systems allow integration of sampling, reagent mixing, and rapid electrophoretic analysis, and also are useful for culturing cells. Pancreatic islets—containing insulin secreting b-cells—have insulin oscillation with 3-5 min frequency or even longer rhythm. Deficiencies in such rhythms have been observed on Type 2 diabetes patients. To continuously monitor single islet insulin secretion patterns would be a helpful approach to understand them.

We have developed a microchip based electrophoresis assay allow such applications. The system incorporates two independent syringe pumps loaded with low and high concentration glucose. By mixing both pumps’ contents—with different flow rates, arbitrary glucose gradients can be generated. Insulin secreted from the islets is measured at 5-10 s intervals by an electrophoretic competitive immunoassay. The integrated system can perform 14000 electrophoresis assays in 24 h and automatically control culture condition. Online calibrations are also incorporated to improve quantification.

The system has been applied to investigate a few projects.

1) Long term secretion dynamics
The islets are alternately perfused with high and low glucose on chip. Preliminary data shows expanded 1st phase response after the 1st glucose period.

2) Effect of toxic agents on secretion such as H2O2 and elevated glucose
The islets pretreated with H2O2 have elevated insulin secretion rather than the normal insulin oscillations. Islets would be cultured in high glucose to find if excess glucose leads to similar results.

3) Effect of incubation conditions on glucose sensitivity
Preliminary results have found that islets pre-cultured in 2.8 mM glucose have slower glucose response than normal cultured islets, but those co-cultured with 100 uM diazoxide have faster response.

The results reveal the versatility of the islet workstation and its use for routine cellular monitoring. It would further be applied for adipocytes-islets interaction studies.
Miniaturization is of interest as a tool for biochemical studies and holds the promise of portable, rapid, sensitive, integrated, and inexpensive analysis for point-of-care diagnostics. Herein, pressure actuated microfluidic devices for electrophoretic separations were developed using soft lithographic techniques. Each device has three-layer poly(dimethylsiloxane) (PDMS) construction. The fluidic layer with a T-shaped design has injection and separation channels. To carry out sample movement the control layer has an on-chip peristaltic pump and four different valves. An unpatterned PDMS membrane layer was sandwiched between the control and fluidic layers. Devices with different valve locations from the T intersection were tested with colored dye by selectively applying the actuation pressure. Currently, we are studying the separation and detection of fluorescently labeled model compounds and a mixture of pre-term birth biomarkers. Future work will involve testing of thermoplastics such as cyclic olefin copolymer or poly(methyl methacrylate) for the fluidic and control layers. We plan to integrate separation with on-chip labeling to develop next-generation microfluidic devices for pre-term birth analysis. This novel, state-of-the-art technology should provide a clinical diagnostic tool to assess pre-term birth, enabling therapeutic interventions.

**Keywords:** Bioanalytical, Electrophoresis, Lab-on-a-Chip/Microfluidics, Sample Introduction

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Heavy metals such as Cd and Pb are threat to human health due to the fact that they are not biodegraded. Although various high sensitive methods such as ICP and AAS have been reported, electrochemical analysis is advantageous since this method requires short time and the system can be miniaturized for on-site analysis. Anodic stripping voltammetry (ASV) has been commonly used for determination ppb level metal ions because the preconcentrate of metal ions on to electrode surface can improve the sensitivity and detection limit. We have previously developed an sp[2] and sp[3] hybrid carbon film electrode by employing the electron cyclotron resonance (ECR) sputtering method. The carbon film electrode was utilized for ASV measurement of Cd at ppb level concentrations.[1] Recently we employed Unbalanced Magnetron sputtering (UBM) method which can fabricated carbon film with similar properties with that formed by ECR sputtering with lower equipment cost.[2] In this work, we optimized sp[2] to sp[3] ratio of UBM carbon film by mainly considering S/N ratio in ASV analysis for Cd and Pb.[3] After optimizing measurement solution and carbon film structure, we incorporated the carbon film electrode in a radial flow-cell and integrated the pre-processing system including pre-column to remove Cu ion and on-line optical decomposition devise to remove organic interferents. With our UBM carbon film electrode, low detection limits of 0.5 ppb and 5 ppb could be archived for Cd and Pb ion, respectively, despite of relativity low current signal due to extremely low noise level compared with GC electrode. With our integrated system, we cloud measure ppb level Cd ion even in the presence of 100 ppb Cu ion and 0.1 mM EDTA.

Reference

Keywords: Electrochemistry, Electrodes, Environmental, Metals
Application Code: Environmental
Methodology Code: Electrochemistry
Cloud point extraction (CPE) is a well-established technique for the pre-concentration of hydrophobic species from water without the use of organic solvents. Subsequent analysis is then typically performed via atomic absorption spectroscopy (AAS), UV-Vis spectroscopy, or high performance liquid chromatography (HPLC). However, the suitability of cloud point extraction for electroanalytical methods such as stripping voltammetry has not been reported. We have developed a CPE procedure for the determination of cadmium (Cd2+) by anodic stripping voltammetry (ASV) with bare and modified glassy carbon (GC) working electrodes. This extraction method was performed without the use of chelating agents, but using iodide to neutralize the charge on Cd2+. This novel combination achieved a detection limit of 1 ppb when using a mercury-coated glassy carbon electrode. Applicability of this procedure to tap and river water samples was also demonstrated. The method can potentially be applied to a wide variety of transition metals as a simple, versatile, and cost-effective detection method.

**Keywords:** Electrochemistry, Extraction, Trace Analysis, Voltammetry

**Application Code:** Environmental

**Methodology Code:** Electrochemistry
The hydraulic fracturing procedure typically involves a high pressure injection of water, a propping agent and different chemical additives into underground bedrock formations for the extraction of oil and natural gas. The chemistry of these fracturing fluids are often a complex proprietary blend designed for a variety of different purposes dependent on the specific type of well being fractured.

The analysis of fracking fluids by inductively coupled plasma optical emission spectroscopy (ICP-OES) may pose difficult challenges. Fracking fluids contain high levels of total dissolved solids which can cause interferences for the analytes of interest. These high concentrations may suppress the signal for the analyte and the internal standard due to plasma loading effects. To minimize plasma loading effects, minimal sample volume (<0.5 mL) was delivered into the system with analysis times of approximately 30 seconds per sample. The minimal sample volume and fast analysis times allowed for high sample throughput and minimal torch devitrification. For extended torch life of fracking samples, a hybrid torch was utilized in analysis increasing torch lifetime approximately 10 times.

Minimal sample dilutions (10x) were prepared to ensure reproducible trace metal detection. Typical precision obtained was ≤2% RSD for 3 replicates. Typical matrix spike recoveries of ±15% were attained and stability over a 300 sample analysis achieved internal standard (Y 361.38 nm) recoveries from 89% to 105%, indicating a robust method with optimized plasma conditions. Additional data visualization was carried out using data analysis software, Tibco Spotfire.
Absract Text

Silver nanoparticles, Ag NPs, are widely used in many consumer products. As a result, they can leach into the environment during product usage, raising concern about their toxicity in the ecosystem. Natural organic matter, NOM, is abundant in water supplies, soil, and sediments, and can bind with Ag+ that is released from Ag NPs. This results in formation of silver-species with altered bioavailability and toxicity. Using fluorous-phase Ag+ ion-selective electrodes (ISEs), we studied Ag+ binding to NOM and showed that Ag+ binding to NOM is favored by a higher pH. Moreover, effect of NOM chemical composition on kinetics and extent of binding was investigated. While only weak Ag+ binding was found for Suwannee River humic and fulvic acid, which are NOM samples with a low sulfur and nitrogen content, stronger binding was observed for Pony Lake fulvic acid, which has higher amounts of these elements. Fast kinetics of Ag+ and NOM binding was confirmed and Ag+ reduction by NOM was shown to cause slow decreases in free Ag+ activity in presence of Pony Lake fulvic acid. More generally, this work has shown that fluororous-phase Ag+ ISEs are effective tools for in situ studying of Ag+ binding to NOM.

Keywords: Dissolution, Electrochemistry, Environmental Analysis, Environmental/Biological Samples

Application Code: Environmental

Methodology Code: Electrochemistry
The use of nanoparticles in consumer products is showing a tremendous increase over time. The National Institute of Standards and Technologies reported that nanotechnology-based consumer products are currently entering the market at a rate of 3 to 4 per week, and it is estimated that $2.6 trillion in manufactured goods will contain nanotechnology by 2014[1].

In spite of their beneficial properties, possible risks for humans and the environment need to be thoroughly investigated, and multiple key characteristics need to be assessed. Detailed information about the quantity, shape, size, size distribution, structure, composition, surface charge and functionality need to be studied and addressed in order to perform an appropriate and reliable risk assessment.

This work presents Single Particle Inductively Coupled Plasma Mass Spectrometry (SP-ICP-MS) as a tool in assessing the fate of engineered nanoparticles in environmental sample types [2,3]. The technique allows for the differentiation between ionic and particulate signals, measurement of particle sizes and size distribution, and assists in monitoring agglomeration.


Keywords: Characterization, Environmental Analysis, Environmental/Water, Nanotechnology

Application Code: Environmental

Methodology Code: Mass Spectrometry
Metal burden in [i]Eudrilus eugeniae[/i] (earthworm) and [i]Pachybolus ligulatus[/i] (millipede) as bio-indicators of toxic metals impaction of soils in Akwa Ibom State, in the Niger Delta region of Nigeria were investigated. The study involved collection of [i]Eudrilus eugeniae[/i] and [i]Pachybolus ligulatus[/i], and their site soil samples from different impacted and non-impacted soils in ten locations spread over four Local Government Areas. Toxic metals (Pb, Fe, Zn, Ni and Cd) in soils and bio-indicator organisms were analysed using Atomic Absorption Spectrometry after acid digestion. Geo-accumulation Index (Igeo) was used to measure the extent of soil pollution, Bio-Accumulation Factor (BAF) to compute the metal uptake from soil by bio-indicators, and multivariate statistics to identify interrelationships among metals and the pollution sources. Metals in all soils were higher than the background levels, and were classified unpolluted to very strongly polluted. Pb, Fe, Zn and Cd were higher in [i]Eudrilus eugeniae[/i] and Ni in [i]Pachybolus ligulatus[/i] at 0.05 level. The organisms absorbed all metals (BAF<1), but [i]Eudrilus eugeniae[/i] accumulated Cd and Fe and [i]Pachybolus ligulatus[/i], Ni and Fe (BAF>1). Metals were clustered into three ([i]Eudrilus eugeniae[/i]) and two ([i]Pachybolus ligulatus[/i]) dissimilar groups. Two principal components extracted each for [i]Eudrilus eugeniae[/i] and [i]Pachybolus ligulatus[/i] accounted for 64% and 72% of total variations respectively. [i]Eudrilus eugeniae[/i] and [i]Pachybolus ligulatus[/i] could be used as bio-indicators in toxic metals impacted soils; and [i]Pachybolus ligulatus[/i] in particular, as indicator of soil Ni pollution due to oil and gas activities in the Niger Delta Region of Nigeria.
In recent years, 1 in 6 Americans have been infected by foodborne pathogens, which each year on average have resulted in ~50 million illnesses, ~130,000 hospitalizations, and 3000 deaths. Preventing distribution and consumption of contaminated foods is challenging because just 100 bacterial cells can rapidly multiply to millions, reaching infectious doses within a few days. Unfortunately, current methods used to detect these few cells rely on similar growth steps to multiply the cells to the point of detection, which also takes a few days. Consequently, there is a critical need for an analyzer that can rapidly extract and detect foodborne pathogens at 100 colony forming units per gram (cfu/g) of food in 2-4 hours (not days), and with a specificity that differentiates indigenous microflora, so that false alarms are eliminated. In an effort to meet this need, we have been developing an assay that extracts such pathogens from food, selectively binds these pathogens, and produces surface-enhanced Raman spectra (SERS) when read by a Raman analyzer. Measurements of E. coli in ground beef, Listeria in cheese and Salmonella in cantaloupe at 100 cfu/g using this assay will be presented.

**Keywords:** Bioanalytical, Food Safety, Portable Instruments, Surface Enhanced Raman

**Application Code:** Food Safety

**Methodology Code:** Vibrational Spectroscopy
Although Surface-enhanced Raman scattering (SERS) is a powerful technique for the sensitive and selective detection of low concentration analytes, the quantitative detection of target in real matrices using an optimal substrate is still a challenge. But the main problem in homogenous SERS immunoassay system is the difficulty in surface modification of nanoparticle. One of the solutions of this problem is to use a thin polymer film to immobilize the high amount of biomolecules.

In the present study, a simple and highly selective homogeneous sandwich assay was developed for fast and ultrasensitive detection of toxins such as SEB and ricin using a combination of aptamer functionalized hybrid magnetic nanoparticles and aptamer immobilized gold nanoparticles as the recognition and surface-enhanced Raman scattering (SERS) component, respectively. The magnetic hybrid particles were first coated with poly(N-acryloyl-L-valine) via surface-mediated RAFT polymerization and then biofunctionalized with appropriate aptamer, which are both specific for target toxin and can be collected via a simple magnet. After separating target toxin from the sample matrix, they were sandwiched with the SERS substrate composed of aptamer and 5,5-dithiobis(2-dinitrobenzoic acid) on gold nanoparticles. The optimization strategies for avoiding aggregation and the analytical performance of the SERS-based assays will be presented.

Keywords: Food Contaminants, Immobilization, Surface Enhanced Raman, Trace Analysis
Application Code: Food Contaminants
Methodology Code: Sensors
Viability of standoff Raman technique is demonstrated by detecting contamination/adulteration in food items like grain flour, olive oil and milk from a distance of several meters. We have characterized standoff Raman technique with reference to parameters like, nature of food item, standoff distance, nature and concentration of contaminants, ability to make quantitative measurements, and sensitivity.

The equipment involves a portable Raman system with a 785 nm laser. The Raman system is coupled to a 2-inch refracting telescope and can be operated in the field either with batteries or a gas-powered generator. This set-up has been used to detect adulteration in powder and liquid-foods.

Results include detection of agrochemicals like fertilizers in wheat-flour. Nitrate contamination can be detected in flour from a distance of up to five meters. Likewise Alum is used for refining flour and has been detected by the standoff Raman technique. Contamination of Olive Oil with cheaper edible oils has been detected from a distance of 1 meter. We have detected Ammonium Nitrate by stand-off Raman technique at distances of up to 100 meters. This serves as a proof of concept that we may be able to detect food contaminants at very large distances.

Stand-off Raman technique can detect food contaminants/adulterants which could be toxic or laced with biological pathogens from a safe, non-contact distance of several meters. The results show a potential to solve issues related to monitoring food-supply chain and characterize the nature of vulnerability.

Keywords: Detection, Food Contaminants, Food Safety, Raman

Application Code: Food Contaminants

Methodology Code: Sensors
The kinetics for the in vitro reactions of acrylamide (AA), a potentially toxic food contaminant formed during elevated-temperature food preparation, with captopril (CapSH), L-cysteine (CySH) and glutathione (GSH), were determined under basic conditions and constant ionic strength (pH 7.10 – 9.10; I = 0.2 mol dm\(^{-3}\), NaCl), and pseudo first-order conditions with respect to AA. The second-order rate constants, \(k\), and activation parameters (\(H^\ddagger\), \(S^\ddagger\), and \(G^\ddagger\)) were determined over the ranges of 293 – 303 K for CySH and 303 – 315 K for GSH and CapSH. Comparison of experimental second-order rate constants at 303 K for CapSH, CySH, and GSH were: 0.13 ± 0.01, 0.34 ± 0.02, and 0.18 ± 0.02 dm\(^3\) mol\(^{-1}\) s\(^{-1}\), respectively and DFT calculations show evidence of diminished intra-molecular hydrogen abstraction reaction in aqueous solution specific only to GSH. An isokinetic plot of \(H^\ddagger\) versus \(S^\ddagger\) yields an isokinetic temperature of 260 ± 24 K and an intercept, \(G^\ddagger\), of 71 ± 4 kJ mol\(^{-1}\) indicating that AA reacts with all three thiols via the similar reaction mechanism. Theoretically determined \(G^\ddagger\) values in aqueous solution (DFT-BVP86/Ahlrichs-TZVP/COSMO-RS) for CapSH, CySH, and GSH, including their zero point vibrational energy, are 212.2, 217.8, and 253.4 kJ mol\(^{-1}\), respectively at 298 K.

Funding for this research was provided by The University of the West Indies, Mona and St. Augustine Campuses

**Keywords:** Amino Acids, Food Safety, Spectroscopy, Toxicology

**Application Code:** Food Safety

**Methodology Code:** UV/VIS
The need for highly selective, efficient protein separations continue to rise due to the costs associated with downstream processing of biopharmaceuticals. Affinity chromatography is considered a fast, simple, highly selective method for biomolecule purification. This is evident in the rise of new affinity ligands including aptamers, antibodies, metal ions, etc. These methods are so useful that biomolecules have been designed with affinity tag modifications to exploit theses powerful interactions. A problem associated with these methods is the cost associated with functionalizing these support phases. Novel lipid tethered ligands (LTL) can allow for a quick and easy method to functionalize hydrophobic surfaces with affinity ligands. LTLs work by strongly adsorbing the lipid to hydrophobic surfaces, while leaving the modified head group exposed in the bulk flow. Biotin functionalized LTL demonstrated to the ability of capturing streptavidin labeled with Texas Red (SAv-TR) while adsorbed to polypropylene capillary-channeled polymer fibers.

A comparative study is underway evaluating the robustness and efficiency of LTLs for the functionalization of polystyrene-divinylbenzene (PS-DVB) micro-beads. PS-DVB packed columns are exposed to biotin functionalized LTL containing solutions of 50% ethanol:water. The functionalized biotin LTL PS-DVB column was evaluated for selective capture of SAv by breakthrough curve analysis. Addition of 0.1% Tween 20 is added to the SAv containing buffer to prevent non-specific binding of the SAv to the PS-DVB beads.

Further evaluations of LTLs will be done to evaluate elution conditions and robustness of PS-DVB columns functionalized with LTLs. LTLs with fluorescent head groups will be used to evaluate conditions necessary to elute the LTLs from the PS-DVB. At times it is easier to separate the ligand from the surface, than overcome the affinity interactions. This is used when regeneration of the stationary phase is easy and cost effective.

Keywords:  
HPLC Columns, Immobilization, Lipids, Separation Sciences

Application Code:  
Bioanalytical

Methodology Code:  
Separation Sciences
Capillary-channeled polymer (C-CP) fibers (polypropylene, nylon and polyethylene terephthalate) have been used as stationary phases for HPLC separation in this laboratory for over a decade. Considerable research has been conducted to employ C-CP fiber stationary phases for downstream protein processing. Protein A modified polypropylene (PP) C-CP fibers have been shown to be a promising affinity phase for efficiently capture of IgG. PP C-CP fibers have been modified by head group-functionalized lipids to introduce different functionalities. Ion-exchange separations are another crucial step in protein downstream processing. In this study, polyethylenimine (PEI), a highly branched and positively charged polyamine, was covalently attached to the surface of polyethylene terephthalate (PET) C-CP fibers. Initial results showed that the PEI modified PET C-CP fiber stationary phases were capable of capture and elution of bovine serum albumin (BSA) under weak anion exchange (WAX) conditions. By choosing the optimized modification process, PEI modified PET C-CP fiber stationary phases showed little reversed phase characteristics, indicating extensive coverage of the hydrophobic surface of native PET C-CP fibers with PEI. The inexpensiveness of PET C-CP fibers and the simple, low-cost modification process make PEI modified PET C-CP fibers a promising choice as stationary phase in protein downstream processing.

Keywords: Bioanalytical, HPLC Columns, Ion Exchange, Liquid Chromatography
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
A heart-cutting two dimensional liquid chromatographic method for the isolation and quantification of amino acid enantiomers from rat brain tissue is demonstrated. Evidence to support the occurrence of D-amino acids in high concentrations in physiological samples has spurred studies toward the elucidation of their importance and function. In this work, the level and regional distribution of select amino acids were determined by achiral-chiral LC/LC. The free amino acid extracts were analyzed as their 9-fluorenylmethyloxycarbonyl derivatives and separated in the reverse phase on superficially porous C18. In the second dimension, enantiomers of selected amino acids were resolved on Chirobiotic-T as it exhibits excellent selectivity. This study focused on D-ser, D-ala, D-leu, D-asp, and D-pro as they hold relevancy as highly functional neurological molecules. Total concentration of amino acids were determined through the use of internal standards and enantiomeric ratios were determined with high sensitivity using fluorometric detection. Because the selected D-amino acids have utility in development and neurotransmission, their potential as disease biomarkers is concurrently being investigated.

Keywords: Amino Acids, Bioanalytical, Chiral, Liquid Chromatography
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Drugs are often reversibly bound to carrier agents such as serum proteins in the circulatory system, causing these drugs to exist in a free or protein-bound form. Because the free drug fraction is generally thought to be the biologically-active form, there is increasing interest in developing methods to estimate the free drug fractions and to study drug-protein interactions in clinical samples. However, commonly-used methods such as equilibrium dialysis and ultrafiltration generally need large amounts of sample and/or long analysis time. Moreover, separate experiments are required to measure both the kinetics and thermodynamics of a drug-protein interaction. To overcome these limitations, a new approach was created based on ultrafast affinity extraction and affinity microcolumns to measure free drug fractions and to study the drug-protein interactions. Human serum albumin (HSA), the most abundant serum protein in the blood, was used as a model soluble protein for this work. Several drugs were examined, such as warfarin, verapamil, chlorpromazine, and sulfonylurea drugs. It was shown this approach could simultaneously determine both the binding and rate constants for drug interactions with soluble proteins. A modified approach based on a multi-dimensional affinity system was also created to simultaneously study the binding of two enantiomers in racemic warfarin with HSA in serum. The same general approach was used to investigate how glycation of HSA, as found in diabetes, may alter the free fractions and affinities of sulfonylurea drugs with normal HSA or glycated forms of this protein. The results indicate that this method can be a powerful tool for the study of drug-protein interactions and can provide information on free drug fractions in serum or in samples with clinically-relevant concentrations of drugs and proteins.
Capillary-channeled polymer (C-CP) fibers have been under investigation and development as stationary phase for high performance liquid chromatography (HPLC) separations for over ten years. They are unique due to the eight capillary channels extending the whole length of the C-CP fiber. This unique shape offers them ~3x greater surface area than that of circular cross-section fibers with the same nominal diameter. When packed into a column, C-CP fibers self-align, yielding a monolith like structure of 1-5 µm open, parallel channels. As a result, C-CP fiber stationary phases exhibit excellent fluid transport properties. A traditionally sized HPLC column packed with C-CP fibers can be operated at high linear velocity (> 100 mm s⁻¹) with low backpressure (< 2000 psi). In this study, a polyethylene terephthalate (PET) C-CP fiber packed microbore HPLC column was sequentially functionalized. The coupling strategy of solid phase peptide synthesis (SPPS) was employed for the attachment of ligands. The common biotin-streptavidin affinity pair was used as a demonstration of the basic methodology, wherein biotin was the immobilized ligand. The subsequent streptavidin binding homogeneity and binding capacity were studied by breakthrough curve experiments and frontal analysis. The biotin functionalized PET C-CP fiber column was shown to be able to affinity capture streptavidin from an enhanced green fluorescence protein (EGFP)-spiked [i]E. coli[/i] cell lysate with high selectivity and efficiency. The SPPS coupling method used here yields PET C-CP fiber stationary phases with great potential for further functionalization by a variety of affinity ligands and used for affinity chromatography applications.

Keywords: Bioanalytical, HPLC, HPLC Columns, Liquid Chromatography
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Capillary-channeled polymer (C-CP) fibers have been studied in this laboratory as stationary phases for protein separations in high-performance liquid chromatography (HPLC). C-CP fibers are uniquely shaped so as to include eight continuous capillary channels which interdigitate once packed into a column. The packed column resembles a monolithic structure of unobstructed flow through capillary channels which reduces backpressure and increases linear velocity, reducing separation time. Fibers are effectively nonporous for macromolecules, resulting in rapid mass transfer and high sample recovery. C-CP fibers made from polypropylene (PP) yield a fairly homogenous hydrophobic surface suitable for reversed phase (RP) chromatography. In a microbore C-CP column, separations can be done very quickly, in less than ten minutes, even at low flow rates, saving time as well as money in column cost, solvent cost and waste generation. Presented is a fast liquid chromatographic separation of a multi-protein suite analyzed by electrospray ionization mass spectrometry (ESI-MS).

Electrospray ionization mass spectrometry (ESI-MS) analysis provides more analytical information than multi-wavelength detectors. However, the low flow rates needed (<0.5 mL/min) for optimal spectral clarity generally imply low linear velocities and slow separations if liquid chromatography mass spectrometry (LC-MS) is desired. To this end, decreased C-CP column diameters (0.5 mm i.d.) are employed to increase linear velocity, and therefore speed separation time, without the need to increase flow rates. Ribonuclease A, cytochrome c, and transferrin were loaded in phosphate buffered saline. Matrix was removed using a water wash before applying a gradient of ACN to elute proteins by hydrophobicity into ESI-MS.
Oxidative stress occurs due to a disturbance of the body’s natural balance between oxidants and antioxidants due to an overabundance of reactive oxygen species (ROS). During a stress event, the overproduction of ROS will disrupt the function of many biological molecules and processes. One example is lipid peroxidation, where ROS attack polyunsaturated fatty acids and result in the secondary production of reactive aldehyde species, such as 4-hydroxynonenal (HNE), malondialdehyde (MDA), and acrolein. These species can then be used as biomarkers of oxidative stress. A method was developed for the separation and simultaneous detection of these three analytes using liquid chromatography with fluorescence detection. The aldehydes were derivatized with dansylhydrazine for 20 minutes at 50°C to form a fluorescent product with a maximum excitation and emission wavelength of 250 and 550 nm, respectively. No other preparation was required before injection. Separation was achieved on a reverse-phase column with a C18-based stationary phase in 20 minutes. This method resulted in LODs of 660 nM for HNE, 1.7 µM for MDA, and 2.4 µM for acrolein. Additionally, this method was used to quantify levels of these reactive aldehyde species in rat urine following a stress event. Epileptic-like seizures were induced in the animals through the use of a chemical agent, 3-mercaptopropionic acid, and the change in concentration monitored. A rise in acrolein concentration after seizures was observed, while HNE and MDA stayed near basal levels. The current method was also validated by comparison to a previously-published method for the detection of malondialdehyde.
Goldenseal has emerged today as one of the 20 most popular herbal supplements used worldwide. The major alkaloids in goldenseal are berberine, hydrastine and canadine, which are believed to be the main bioactive compounds with various pharmacological effects. Most previous researchers have used LC-UV methods to quantify berberine, canadine and hydrastine in goldenseal. LC/MS techniques are extremely powerful tools for the quantification of alkaloids in medicinal herbs. Quantification using LC/UV or LC/MS techniques requires the standard compounds to have a known purity to construct calibration curves. The quantitative high-resolution proton nuclear magnetic resonance method (1H-qNMR) method is rapid, highly specific, linear, can provide traceability through use of well characterized, internal or external standards of very high purity.

In this study, the 1H-qNMR method has been developed with external standards to determine the purity of berberine, canadine and hydrastine, which are used for quantification of those compounds in goldenseal extract. We have developed a LC-MS/MS using selected reaction monitoring (SRM) to quantify these three alkaloids in goldenseal extracts. The purity results of berberine, canadine and hydrastine are 93.35; 99.36 and 99.32% (respectively). To date, berberine, canadine and hydrastine have been quantified at mg/g levels powdered goldenseal extract.

In conclusion, the current 1H-qNMR and LC-MS/MS using SRM option methods are rapid, highly specific and can assign the purity of standard compounds and quantify the three major alkaloids of goldenseal root and will provide a useful tool in understanding the bioactivity of this medicinal plant.

Keywords: Liquid Chromatography/Mass Spectroscopy, Magnetic Resonance, Natural Products, Quantitative
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
### Abstract Text

The chemical industry: a data industry. Yes, we make products, tens of thousands of them, from the fuel that powers our cars, to lifesaving medicines, to key components of the food we eat every day. We transform raw materials into the products that make modern society possible; it’s pretty amazing when you think about it. But in each company, in every chemical sector, we also make and manage vast amounts of data that underpin the discovery, development, manufacturing, quality, safety and efficacy of our products.

It all starts with the data. No data, no products. And our data is in a sad state.

We live in a world where millions of us can find any song we want on our phones and then listen to it from our car stereo in seconds. Yet finding and assembling data to support a scientific decision, regulatory or legal query can take hours, days, weeks, or longer. We can share our thoughts, a song, a playlist or pictures with our friends in seconds, yet sharing scientific observations, a fundamental scientific endeavor, is inefficient, hindered or sometimes impossible.

What’s going on?

Bluetooth, WiFi, MP3, HTTP, and HTML are standards we use nearly every day because our devices (e.g., phones, cars) seamlessly implement these standards using software toolkits like those provided by iOS, Windows and Android. You don’t even think about the standards– the toolkits do the heavy lifting. Yet no such common standards or software toolkits exist for our devices at work, our laboratory instruments.

In this talk we describe a software toolkit being created to embed standards for analytical data. We also discuss why this approach addresses fundamental root causes of our data challenges rather than treating symptoms of the problem. Finally, we describe how such a community-driven approach can transform how we create, use, share and manage scientific data to create innovative products; how it will simplify and enhance productivity in our industry. After all, we are a data industry.

**Keywords:** Data Mining, Informatics, Scientific Data Management, Software  
**Application Code:** Consumer Products  
**Methodology Code:** Laboratory Informatics
# A Disruptive Technology Platform for Configurable LIMS

Laboratory Information Management Systems (LIMS) have evolved significantly over several decades to help end-users achieve their lab management requirements. Some LIMS platforms have provided very flexible systems in terms of configurability, but the reality is that most of the systems still need to be customized to meet specific end-user requirements. This customization, whatever technology is used, always involves some degree of programming. The programming tools or integrated development environments within LIMS platforms mostly require vendor tool specific programming skills. Even an experienced programmer still needs to know the programming language and the underlying database structure in order to make changes or modifications to the system. This leads to increased total cost of ownership and validation challenges.

Now for the first time in the LIMS world there is a fully developed non-programming solution. QuaLIS Studio allows users to make changes to the LIMS behaviour, to the business rules it uses and to create completely new functional capability without writing code. Additionally it has minimal requirements to understand the underlying database structure. QuaLIS Studio is designed using object oriented technology which simply allows users to drag and drop user interface components and interact with server-side class objects which in turn perform transactions. Extra functionality can be added by re-using existing class objects or by creating new classes using the business rule definition tool which in turn generates the underlying code for the server-side class. QuaLIS Studio provides a significant speed increase for customization and does not require an IT professional to implement modifications and enhancements to the system. Regulated industries will benefit from using this new programming-free approach and all industries will benefit from the speed and ease of configuration without customization.

## Keywords
- Informatics
- Laboratory Informatics
- Software

## Other Information
- **Application Code:** Other
- **Methodology Code:** Laboratory Informatics
There are a lot of assumptions around chemistry labs that have shaped the way that these facilities have been designed for decades, but some of these assumptions are now coming back to haunt us. There is a growing trend among research institutions to renovate or replace their existing chemistry labs. We are seeing these spaces being moved out of the upper floors of research towers. Old unsafe facilities are being replaced and inefficient spaces are being overhauled. New chemistry teaching and research pedagogies are changing the program requirements and new technologies are allowing us to challenge the old assumptions and build energy efficient spaces that are safe and flexible.

In this presentation we will look at assumptions around safety, program, building codes, equipment loads, ventilation rates and, of course, fumehoods. We’ll investigate what are the possible payoffs for challenging them and what are the costs for not challenging them. We will look at the new technologies that are available and see which are safe and can save you money and which will be a waste of your facilities dollar.

Key Points
1. Safety is paramount in chemistry labs. We have learned a significant amount about the dangers posed by hazardous chemicals over the past few years and this is now reflected in building codes and the plan review process.
2. Reducing CFM doesn’t have to mean reducing safety. Targeting where how and when you reduce your flows can actually make your lab safer.
3. Chemistry labs don’t have to be energy hogs if everyone works together from the beginning and challenges the assumptions.

Keywords: Industrial Hygiene, Lab Management, Laboratory
Application Code: Laboratory Management
Methodology Code: Other
For decades helium ionization detectors have been the detector of choice for the analysis of high purity and ultra high purity gases for atmospheric impurities. In some cases these detectors have not evolved since their inception, others have become obsolete, but those designs are for a bygone era, when the cost and availability of helium was not a concern.

However, with greater industry demand for helium, changes in the politics of helium, global supply chain risks, rising prices, and a growing international awareness of the limited supply of helium, it is both economically and environmentally responsible to consider how this resource can be used more efficiently and to consider options that exist which can greatly reduce helium consumption and perhaps to recycle as a means of further reducing waste and cost. This presentation will provide comparisons of older techniques and emerging methodologies, and where the greatest opportunities for improving analysis, reducing time and helium consumption all of which help in reducing cost of ownership, while simultaneously improving our stewardship of one our limited environmental resources.

We will compare detector systems, chromatographic techniques and system considerations which can drastically reduce your helium footprint.

**Abstract Text**

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However, with greater industry demand for helium, changes in the politics of helium, global supply chain risks, rising prices, and a growing international awareness of the limited supply of helium, it is both economically and environmentally responsible to consider how this resource can be used more efficiently and to consider options that exist which can greatly reduce helium consumption and perhaps to recycle as a means of further reducing waste and cost. This presentation will provide comparisons of older techniques and emerging methodologies, and where the greatest opportunities for improving analysis, reducing time and helium consumption all of which help in reducing cost of ownership, while simultaneously improving our stewardship of one our limited environmental resources.

We will compare detector systems, chromatographic techniques and system considerations which can drastically reduce your helium footprint.
Abstract Text
We will deliver an analysis of the critical components of a company's overall enterprise manufacturing intelligence (EMI) strategy, plan, structure backed by real cases. We will delve into the criteria that impacts manufacturing operations and the requirements involving the real-time analysis of data from any and all processes in order to react with quick, educated decisions to reduce costs, increase yields and improve quality.

Manufacturers, LIM providers and LIM users will learn how to use enterprise manufacturing intelligence (EMI) and integrate, analyze and visualize information from data sources across operations for better, faster results. LIMs Providers and Users of LIMS will get an in-depth understanding and the resulting impact of using mission-critical data that is collected and managed by LIMS as part of the greater EMI environment.

EMI the fastest growing technology investment segment in manufacturing according to analysts. LIMS managers need to be proactive to ensure their LIMS is a key part of the overall EMI plan. By getting involved now, laboratories greatly mitigate the risk of having integration plans dictated to them or worse - their companies start considering alternatives to LIMS. This session will explain the role of LIMS in the overall EMI environment, best practices integrating LIMS with other process information systems, and the importance of taking a leadership role in an EMI roll-out.

Keywords: Data Analysis, Data Mining, Informatics, Laboratory Informatics
Application Code: Laboratory Management
Methodology Code: Data Analysis and Manipulation
A recent revolution in chromatography is the development of porous polymer monoliths (PPMs) as stationary phases. Compared to traditional silica-based stationary phases, PPMs have very different structures. Monoliths can be customized by changing polymerization conditions, porogens, or monolith components. Small changes in polymer recipes may result in dramatically different structures and separation performances. A lauryl acrylate porous polymer monolith was synthesized in fused silica capillary columns with different linkers, with and without an anionic electroosmotic flow modifier. Cross-sections of polymers were coated with gold or platinum and imaged using secondary electron SEM in order to provide morphology information for better understanding of the behavior of the lauryl acrylate PPM. The highly porous, interconnected network of globular polymer clusters enable the mobile phase and analyte to interact. The clusters are randomly shaped, however, the globular particles are similar in size and thus used for quantitative comparison. To eliminate imaging artifacts, commercially calibrated nanoparticles were imaged in situ with our polymers. These high-resolution SEM images enabled direct visualization and comparison of different polymer compositions. Measurements of particle size and edge thickness were also taken from images and analyzed statistically. The results provide insights for understanding the relationship between structure and properties of lauryl acrylate porous polymer monoliths.

Keywords: Microscopy, Statistical Data Analysis, Surface Analysis
Application Code: General Interest
Methodology Code: Microscopy
A separation of rhodamine laser dyes is accomplished through the use of an electrospun polymeric nanofiber mat acting as the stationary phase in planar electrochromatography (PEC). A solution composed of silica nanoparticles and polyvinylpyrrolidone (PVP) was dispersed/dissolved in reagent alcohol and electrospun to create a mat of nanofibers. The nanofiber mat was then cross-linked to reduce the solubility of PVP in common solvents used in mobile phases. The silica/PVP mat was previously shown as an effective stationary phase in ultra-layer chromatography (UTLC). Cross-linking parameters were studied to show chromatographic performance with optimization under different reaction conditions. The influences of ternary mobile phase conditions, applied potential, and development time on analyte migration distances were studied. The laser dye separation utilizing PEC will be compared with UTLC analysis in terms of efficiency and analyte order of retention. Time of analysis is decreased for PEC (60-120 seconds) and occurs faster than in UTLC separations.

This research was funded by the National Science Foundation.
Monolithic stationary phases include both silica and polymeric types. Both of these phases behave differently from traditional particle based stationary phases. We are examining the behaviour of organic, porous polymer monoliths used in capillary electrochromatography so that these materials and their performance as stationary phases can be better understood.

In order to compare the performance of porous polymer monolithic stationary phases to traditional stationary phases, the relationship between analyte diffusion and retention is explored and modeled with Eq. 1, first published by Knox and Scott in 1983.

\[
Deff = \frac{(mDm + k)}{(1 + k)} \quad \text{Eq. 1}
\]

\[
(1 + k) \quad Deff = \frac{(mDm + k)}{(sDs)} \quad \text{Eq. 2}
\]

This relationship links \(Deff\), analyte diffusion in the presence of a stationary phase; \(Dm\), the diffusion coefficient of the analyte in the mobile phase; \(m\), the column obstruction factor; \(sDs\), the kinetic parameter; and \(k\), the retention coefficient. When rearranged as is shown in Eq. 2, this relationship predicts a linear result if \(Deff\) is plotted versus \(k\). However, literature examples of studies of traditional stationary phases show negative and curved deviations from the predicted linear relationship.

We are applying this approach to studies of a lauryl acrylate porous polymer monolith (PPM). Diffusion studies were carried out with a homologous series of alkyl benzenes (toluene through 1-phenyl octane). The lauryl acrylate PPM was prepared in situ as stationary phase in a capillary column. The peak parking method was used to measure the axial diffusion of analytes (\(Deff\)) within this stationary phase at after allowing the analytes to diffuse for various times. The rate of diffusion in mobile phase (\(Dm\)) was also measured in an open column. After treating the data according to Eq. 2, a linear relationship was obtained. Our results show significantly less deviation from an ideal Knox plot compared to literature reports of other stationary phases. This linear relationship is due to the very low internal porosity which results from the unusual structure of the polymer monolith. Characterization of this new stationary phase on a fundamental level provides useful information for its development.

**Keywords:** Capillary LC, Chromatography

**Application Code:** General Interest

**Methodology Code:** Separation Sciences
We have recently reported the preparation of microfabricated, carbon nanotube (CNT)-templated SiO$_2$ nanowire thin layer chromatography plates. One of the principle challenges in this work has been to find a suitable method to conformally coat carbon nanotubes with the desired stationary phase material. Here we present an overview of our fourth microfabrication process. In this development, true atomic layer deposition (ALD) in commercially viable equipment is used to coat the CNTs with SiO$_2$ for the production of robust plates capable of performing rapid, high-efficiency, normal phase separations.

**Keywords:** Materials Characterization, Modified Silica, Surface Analysis, Thin Layer Chromatography

**Application Code:** High-Throughput Chemical Analysis

**Methodology Code:** Separation Sciences
The work presented in this talk evaluates silicon nano-pillars for use as planar chromatographic platforms. Electron beam lithography (EBL) and Dewetting (DW) protocols were used to create nano thin layer chromatography (NTLC) platforms. With these wafer level fabrication methods we are able reduce the size of separation medium beyond ultrathin-layer chromatography (UTLC). The pillar heights for all systems investigated are 1-2 micron. The pillar diameter/pitch is 400/550-700 nm for the EBL arrays. The stochastic DW systems have an average pillar diameter/pitch of 230/640 nm. A porous silicon oxide layer (25 nm) was added to each of the pillar array types and then a C18 stationary phase created. Flow was studied and the values for the permeability constant (K0) for the EBL cases were ~ 8x 10^-7 and for the DW arrays ~5x 10^-7. These values are slightly better than our prior report for UTLC pillar arrays. The experimental plate height using Sulforhodamine test dye was ~ 0.1 micron and reproducible for an EBL platform. However, this was done with a slow-evaporating benzyl alcohol mobile phase and there was probably considerable band focusing during the drying process. The same test dye yielded a plate height of ~ 1 micron with the DW array when a more traditional solvent system was used. Separations of fluorescent dyes, environmentally significant derivatized-amines, and anti-tumor drugs are illustrated.

This research is supported by the National Science Foundation under Grant CHE-1144947 with the University of Tennessee and a portion was conducted at Oak Ridge National Laboratory.

Keywords: Materials Science, Nanotechnology, Separation Sciences, Thin Layer Chromatography
Application Code: Nanotechnology
Methodology Code: Separation Sciences
Nanoporous gold (NPG) is an attractive material for such applications as catalysis and immunoassays due to its ease of fabrication and large surface area density. The nano-scaled features and bicontinuous geometry, however, suggest that NPG might also find practical use as a membrane towards (bio)molecular separations. The small constrictions not only demonstrate size-selective behavior, but also promote electroosmotic flow (EOF) effects through the pores. Furthermore, the amenability of Au surfaces towards self-assembled monolayer (SAM) formation allow for precise control over of the surface charge of the NPG. In effect, dynamically tuning the strength and direction of EOF is possible through modulation of this surface charge.

Herein, NPG was prepared through free-corrosion de-alloying of an Au-containing alloy with a concentrated nitric acid bath. Average pore sizes of 50 nm were obtained for the as-prepared NPG, as determined using scanning electron microscopy. The pore sizes were tuned to a desired average diameter by electrodepositing Au monolayers following a surface limited redox replacement approach. Further modifications to the surface charge of NPG were made using standard SAM techniques by immersing the NPG in various omega-functionalized alkanethiol solutions. A custom reservoir assembly coupled to a UV-visible absorption spectrometer was used to monitor the translocation of tracer molecules, such as sodium benzenesulfonate and methyl viologen in the presence of an electric field. The surface charge and pore size was found to have a direct effect on the rate at which these analytes transited the NPG membrane.

Support for this work was provided through the University of Illinois at Urbana-Champaign and the National Science Foundation Graduate Research Fellowship Program (NSF DGE-1144245).

Keywords: Electrochemistry, Materials Science, Membrane, Nanotechnology
Application Code: Materials Science
Methodology Code: Separation Sciences
Porous polymer monoliths (PPMs) are recently developed new stationary phases that have many advantages over traditional phases. However, fundamental characteristics of these polymers haven’t been fully studied. This work examines the thermodynamics of analyte retention on lauryl acrylate PPMs used as stationary phase for capillary electrochromatography (CEC).

For an alkyl benzene series (toluene through octyl benzene) of analytes, the effect of temperature on retention was examined using CEC. Temperatures ranging from 25 °C to 60 °C in 5 °C increments were tested and a clear and expected trend was obtained indicating a reduction in retention as temperature increases. Thermodynamic parameters such as entropy and enthalpy were derived from the Van’t Hoff equation.

While the enthalpy of retention is taken from the slope of a Van’t Hoff plot, determination of the entropy of retention relies on obtaining the value for phase ratio. Nanoflow HPLC was used to measure the volumes of mobile and stationary phases. It is also crucial to determine if the phase ratio is a function of temperature. Precise measurements of the flow rate enabled accurate calculation of phase ratios at a variety of temperatures. These studies were expanded by varying the amount of acetonitrile in the mobile phase. In addition, columns with different linkers, with and without anionic electroosmotic flow carriers were investigated and compared.

**Keywords:** Capillary LC, HPLC, Separation Sciences, Thermal Analysis

**Application Code:** General Interest

**Methodology Code:** Separation Sciences
New Chromatography Stationary Phases

Polyionic Ionic Liquid GC Stationary Phase Evaluations

Ionic liquids are a class of nonmolecular ionic solvents with low melting points. These liquids are unique combinations of cations and anions and can provide a variety of different selectivities when used as stationary phases in capillary gas chromatography. The majority of the polyionic ionic liquid phases that we have been evaluating all provide polar and highly polar selectivities similar to polyethylene glycol based biscyanopropylpolysiloxane phases. These phases will provide unique selectivity for the evaluation of a number of variety of different samples including petrochemical, environmental and food and beverage. The purpose of our studies is to determine the effects changing the cation and spacer groups on the selectivity and stability of the phases. Selectivity was determined and compared using various isothermal and temperature programmed test mixes. Particular cation and anion combinations appear to provide very unique selectivity by the shifting of normal alkane relative to aromatic and other chemical species. New combinations of cations and anions have been evaluated which provide unique selectivity and stability to the phases. We will also demonstrate the effects of the various functional group combinations on the overall stability of the ionic liquid stationary phases.

Keywords: Capillary GC, Gas Chromatography
Application Code: Other
Methodology Code: Gas Chromatography
Water and alcohol are found in many pharmaceutical products, both are used for their solubilizing properties or are residuals from the synthesis process, in addition water is absorbed due to the hydroscopic nature of the compounds, whereas alcohols are added for their antimicrobial properties. Since one or both of these are found in tablets, syrups and excipients they must be measured, monitored and controlled. Moreover, water content can lead to microbial growth, hydrolysis and affects dosing. Alcohols while important to solubilizing compounds both as intermediates and end products can have harmful effect and undesirable interactions with other drugs. Current analytical methods have difficulties due to the wide varieties of drugs functional groups. There is also a lack of a method which is able to simultaneously detect both water and a range of alcohols. Karl Fischer titration (KFT) is used to detect water due to it being precise and specific to water, however its specificity means it is unable to measure alcohols. In addition, KFT is known to have solubility issues and side reactions. The ethanol content is determined by a few different methods, density measurements, distillation, or refractive index depending on the state of the sample. These methods are time consuming, not sensitive for trace analysis of ethanol or require benzene or toluene extractions. We have developed an easy, fast and accurate headspace gas chromatographic method to simultaneously determine the water and alcohol concentrations. This method works for both liquid and solid samples and does not have side reactions with the working media.

Keywords: Gas Chromatography, Headspace, Pharmaceutical, Water
Application Code: Pharmaceutical
Methodology Code: Gas Chromatography
The detection and quantification of residual solvents present in clinical and commercial pharmaceutical products is necessary from both patient safety and regulatory perspectives. Head-space gas chromatography is routinely used for quantitation of residual solvents for small molecule APIs produced through synthetic processes; however residual solvent analysis is generally not needed for protein based pharmaceuticals produced through cultured cell lines where solvents are not introduced. In contrast, Antibody Drug Conjugates and other protein conjugates where a drug or other molecule is covalently bound to a protein typically use solvents such as N,N-dimethylacetamide (DMA), N,N dimethylformamide (DMF), dimethyl sulfoxide (DMSO), or Propylene Glycol (PG) to dissolve the hydrophobic small molecule drug for conjugation to the protein. The levels of the solvent remaining following the conjugation step are therefore important to patient safety as these parental drug products are introduced directly into the patient’s bloodstream. We have developed a rapid sample preparation followed by a gas chromatography separation for the detection and quantification of several solvents typically used in these conjugation reactions. This generic method has been validated and can be easily implemented for use in quality control testing for clinical or commercial bioconjugated products.

Keywords: Bioanalytical, Biopharmaceutical, GC, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Gas Chromatography
Analytical Method Development and Robustness Evaluation for Gas Chromatographic Analysis of Piperazine Designer Drugs

Developing a method to analyze and detect piperazine compounds (ca. BZP and TFMPP), will aid in the screening for emerging synthetic piperazine drugs on the market. The objective of this research is to optimize the conditions and parameters for a method to detect and screen for piperazine-derived compounds, by using gas chromatography (GC) coupled to mass spectrometry (MS), a flame ionization detector (FID) and a nitrogen phosphorus detector (NPD). Due to the amine group of the piperazine, an NPD has higher sensitivity than a FID and a single quadrupole MS system, therefore providing a lower limit of detection. Additionally, the NPD is likely to exhibit less instrumental drift than the MS, potentially making it a better choice for quantification.

A challenge to the analysis of piperazines is their chemical reactivity in the GC sample pathway. A series of commonly used piperazine drugs were evaluated as part of a study to investigate the chemical reactivity. During the course of this research, the impact of the deactivation chemistry of the inlet liners and columns was evaluated, with results ranging from poor to excellent. This method increased the accuracy of the analysis by comparing the chemical reactivity in the GC pathway and increased the sensitivity and selectivity by coupling MS, FID and NPD. This complete investigation of the chromatographic variables as directed to both native piperazines, and their relevant metabolites, in both recreational drug samples and human urine will allow for a consolidated analytical methodology that is more efficient.

Keywords: Drugs, GC Detectors, Method Development
Application Code: Clinical/Toxicology
Methodology Code: Gas Chromatography
In an effort to increase the mass loading of crude phyto-extracts in a relatively rapid fractionation process, we are fabricating a large-scale spiral countercurrent chromatograph. The scale of instrument will be suitable for the research laboratory and the parameters determined in this study will be applied to further scaling to pilot and even larger process. Equally important is to develop the various solvent systems to be easily applied for all types of natural product molecules. In the last few years, spiral separation columns (rotors) have been discovered that can retain much better the stationary phases of all 2-phase solvent systems than other separation column-coils utilized in planetary centrifuges of countercurrent chromatography. A new bench-top chromatograph carrying 2 balancing spiral tubing support rotors (20-cm OD) with 2.1 mm ID FEP tubing will be operated at increased RPM. New active metabolites such as flavonoids and glycosides will be isolated from extracts of Lessertia frutescens and Harpagophytum procumbens plants of So. Africa. Solvent systems will be designed to maximize the isolation and recovery of targeted compounds in a separation.

Funded by 1-R43AT008296-01, SBIR Phase I grant from the NCCAM Institute of the NIH.
In this presentation we introduce some new approaches on the development of new methods for drug screening of active components from Chinese medicine in our research group [1-3]. We have successfully developed a capillary electrophoretically mediated microanalytical technique for screening aromatase inhibitors from Chinese medicines [2] and an neuraminidase-immobilized enzyme microreactor for screening of neuraminidase inhibitors for anti-avian influenza virus from Chinese medicines by capillary electrophoresis. In addition, these novel methods can also be well used for studying the molecular interaction between drug and target enzyme and measuring the enzyme dynamic parameters such as Michaelis constant. Injection procedure of EMMA and electropherograms for screening the aromatase inhibitors are shown in Figure A and B.

Reference:
3. Haiyan Zhao and Zilin Chen*, Biomicrofluidics, 2014, Submitted

Acknowledgment--This work was supported by National Natural Science Foundation of China (No.21375101), Doctoral Fund of Ministry of Education of China (No 20110141110024), the Hubei Provincial Scientific Foundation (No 2011CDB475) and Wuhan Science and Technology Bureau (No: 20140601010057).
It is well known that it is necessary to separate enantiomers in order to show the true effectiveness of chiral drugs. Often, especially at the early stage of drug discovery, it is necessary to quickly separate enantiomers in order to evaluate the pharmaceutical activity of a specific candidate. Among all possible separation tools, capillary electrophoresis (CE) is by far the best and fastest method of choice for this purpose. The primary reason behind this is the combination of the high resolving power of CE and the availability of a wide choice of chiral selectors (CSs), especially cyclodextrin (CD) based CSs including [alpha]-, [beta]- and [gamma]-CDs. However, it is hard to pick and choose specific CDs as the selectivity of those individual CD based CSs are unknown. Thus, it is necessary to further develop new CSs that can offer a quick and fast separation mechanism for quick and dirty start work. For this reason, we have developed a new class of CSs using various sulphated CDs as the constituents to form a polymer CSs. On the basis of polymerizing sulphated [alpha]-,[beta]- and [gamma]-CDs, we further developed mixed CDs as well as other CSs to form unique polymers. We will present our results from synthesis of those new CSs and the separation results for chiral drugs.

Keywords: Capillary Electrophoresis, Chiral Separations, Cyclodextrin, Separation Sciences

Application Code: Pharmaceutical

Methodology Code: Separation Sciences
Incidents involving release of chemicals into the environment often require rapid response, which includes testing to determine the extent of the contamination. Sometimes there are no established environmental test methods for the chemicals involved. For example, the chemical spill which took place in the Elk River in West Virginia in 2014 involved two chemicals, 4-methylcyclohexane methanol (MCHM) and propylene glycol phenyl ether (PPH) which are not included on compound lists of any US EPA test methods. One approach in testing for odd compounds such as these is to use pieces of conventional methods such as purge & trap, soxhlet and liquid/liquid extraction techniques. An alternative approach to consider is solid phase microextraction (SPME), a sampling technique which was commercialized in the early 1990’s. It has found utility in the testing of a wide variety of compounds from many different types of sample matrices. SPME is a fast, easy, and highly sensitive technique which uses no solvent, making it “greener” than conventional methods. It can be used for field sampling in some cases, and when combined with GC-MS analysis, for non-routine testing such as environmental forensic investigations, or screening related to chemical releases. We will present data showing the application of SPME to non-routine environmental testing. Its utility to the extraction of MCHM and PPH from water will be presented as well as other applications.

Keywords: Environmental/Water, Sample Preparation, SPME
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Environmental laboratories have been pushed to the limit to keep costs low since a downward price spiral in the 1990’s made profits hard to maintain. The market contraction has made it very difficult for laboratories to do much beyond that required for the tasks at hand, although some laboratories have been able to focus on a greener approach to their work.(1)

Recent changes have caused laboratories to take a second look at air emissions, mostly arising from solvent evaporation of organic extracts. Through Title V and State Implementation Plans up to 10 tons of chlorinated solvent and up to 25 tons total of solvent may be emitted with a valid permit. For extremely small emitters, no permit is required if they are not at risk of exceeding the limit. Some states, such as Massachusetts have reduced the limit to one ton and recently found several laboratories were exceeding this and fined them. (2)

This paper will talk about several ways to reduce solvent emission, including reducing sample size to reduce the solvent amount that may be required for extraction. Since chromatographic techniques have improved tremendously over the years, the full capability of the chromatograph is not taken advantage of with older environmental methods. The use of solid phase extraction (SPE) can also be used to reduce the solvent needed for elution. Solid phase extraction is well established and offered as an alternative to liquid-liquid extraction in many US EPA methods. The third technique to help reduce solvent emissions is recapture of the solvent after the evaporation step. The requirements for this process will be examined and the feasibility evaluated.


Keywords: Air, Environmental, Solid Phase Extraction, Solvent
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Large temporal and spatial variability of the concentrations of air contaminants makes current health risk assessment practices questionable because of the short duration of air sampling. The best way to improve the quality of risk assessment is to collect data on pollutant concentrations over longer periods, either through multiple sampling events, or through sample collection over a long time. Such procedures would provide better estimates of the long-term average concentrations, and thus better evaluation of health risks. Passive sampling is capable of meeting the above requirements in principle, yet quantitative passive sampling of volatile organic compounds from air lasting more than a few weeks has never been reported. In this study, the suitability of a modified version of a permeation passive sampler known as the Waterloo Membrane Sampler (WMS) for long-term sampling has been evaluated.

The study showed that a simple modification involving increasing the permeation membrane thickness from 0.75 µm to 1016 µm made it possible to use the WMS to collect long-term VOC samples from humid atmospheres over prolonged periods. Six different sorbents were tested (Anasorb 746, Tenax TA, Carbopack B, Dowex Optipore, XAD-2, Carboxen 1016) to reveal the differences in the strength of each sorbent towards specific analytes. The results indicated that WMS filled with Anasorb 746, Tenax TA or Carbopack B could provide 3-month time-weighted average concentration measurements for most compounds of interest. For these samplers, good correlations between linear temperature-programmed retention indices and the calibration constant values were obtained, as predicted by the theory. In addition, a good agreement between experimental and theoretical uptake rates, as well as low variation coefficients within and between months, were achieved. Consequently, a WMS customized in this way could be an invaluable tool for better assessment of risks associated with VOCs present in air.

Keywords: Environmental Analysis, Gas Chromatography, Membrane, Sampling
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
The new SPME Arrow represents a promising alternative to traditional SPME instrumentation, offering mechanical robustness and increased sorption phase volumes for improved sensitivities. While all commercially available SPME Fiber coatings may be realized as well for SPME Arrow, phase volumes of up to 15.3 µL in comparison to 0.61 µL with traditional 100 µm SPME-fibers enable an improved performance and potentially novel fields of application. The required changes to the utilized analysis-system are marginal, allowing injector-penetration by the larger diameter of the SPME Arrow (1.15 - 1.5mm). The specially designed tip of SPME Arrow minimizes septum wear to levels below traditional SPME-fibers.

In the presented results, we compare the new SPME Arrow with traditional SPME-fibers using polycyclic aromatic hydrocarbons (PAH) as analytes, PDMS as sorption phase and GC/MS detection. Reported literature results typically contain calibrations down to 10 ng L-1 with satisfactory linear correlations and reproducibility in higher concentration ranges.

Our results demonstrate how sensitivity and reproducibility benefit from the increased sorption phase volume of SPME Arrow, enabling calibrations down to the pg L-1 range, even in real-world groundwater matrices. Furthermore, the enhanced mechanical reliability of SPME Arrow is a considerable benefit to the robustness of the overall analytical technique.

We acknowledge the support by the following companies: CTC Analytics AG (Zwingen, Switzerland), BGB Analytik AG (Adliswil, Switzerland) and Shimadzu Europa GmbH (Duisburg, Germany)
Nitric oxide (NO) and calcium ion (Ca[^2+]) are important biological active species in regulating fundamental physiological functions. NO is continually produced by various NO synthases (NOS) from cells under normal condition. The activity of constitutive NOS (cNOS), which is one of the NO synthases, is known to be dependent on Ca[^2+] ion and calmodulin. Due to the close linkage between NO and Ca[^2+] concurrently in living biological system. In this presentation, we demonstrate the simultaneous real-time NO and Ca[^2+] measurements by using electrochemical dual microsensor. A NO/Ca[^2+] dual microsensor possesses two working electrodes (WEs) and two Ag/AgCl counter/reference electrodes for each working electrode: one porous Pt micropore-disk electrode (diameter of disk 25 [micro]m, WE1) is used for amperometric NO measurement and the other Ag microdisk electrode modified with ionophore membrane (diameter of disk 76 [micro]m, WE2) is used for potentiometric Ca[^2+] measurement. For a sensor feasibility test, the prepared dual sensor is applied for real-time simultaneous measurements of the dynamic change of NO and Ca[^2+] levels in living rat organs (e.g., brain, kidney). This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning(2014R1A2A2A05003769).
Stochastic nanopore sensing, a resistive-pulse, single molecule detection technology, presents a promising strategy to develop biosensors for quantitative measurements of target analytes. Specifically, the utility of bio-inspired nanopore sensors has found significant use in a variety of applications, including nanomedicine, environmental monitoring, nanoelectronics, and sensing. However, most biological nanopore-based sensors have been limited to the utility of well-behaved protein channels with open conductance states, but lack chemical specificity to target molecules of interest. In this study, we report on the use of the ion-channel activity of heat shock cognate 70 (Hsc70) as a means to specifically and quantitatively detect ATP. Hsc70 reconstitutes into artificial lipid bilayers and forms multi-conductive, ATP-regulated ion pathways, but does not remain in an open, conducting state; rather, requires ATP to induce formation of ion pathways through an artificial membrane. To account for this multi-conductance behavior, we introduce the measurement of ‘charge flux’ to characterize the ATP-regulated channel activity of Hsc70, which enables sensitive quantification of ATP (100 nM - 4 mM) using a traditional bilayer chamber set-up. Furthermore, we demonstrate the ability of Hsc70 as a new biorecognition element in developing an ATP-specific, nanopore-based biosensor supported on a glass nanopore (GNP) membrane, which shows comparable sensitive detection of ATP in the millimolar range (1 - 4 mM). This provides the very first demonstration of the suitability of the GNP biosensing platform in performing small-scale ATP measurements using a naturally occurring pore with high specificity for ATP. We believe that this new class of biosensors will present a rapid and simple analytical methodology in detecting ATP with unprecedented sensitivity, specificity, and spatiotemporal resolution; thus, will add to the growing toolbox enabled by biological nanopore-based sensors.

Keywords: Bioanalytical, Biosensors, Electrochemistry, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Sensors
Sensors - Electrochemical Platforms (Half Session)

Detection of Total Antioxidant Concentrations Using a Nanocarbon Paste Electrode

The electrocatalytic reaction between catechol and the antioxidants, glutathione, cysteine, homocysteine and ascorbic acid is studied at a nanocarbon paste electrode and used to measure the total antioxidant concentrations in aqueous solution. Two different approaches were studied: one in which catechol is dissolved in solution and the second in which catechol is dissolved into the nanocarbon paste electrode. Similar limits of detection of 2.0µM and 1.9µM and sensitivities of 8.8x10^{-3}µA/µM and 0.11µM^{-1} are reported, respectively at nanocarbon and nanocarbon-catechol paste electrodes. Three different commercial multivitamin drug samples were analysed and the results were in a good agreement with those from independent analysis.

ACKNOWLEDGEMENTS
This work was supported by FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), Capes (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and Propesq-UFJF (Pró-reitoria de Pesquisa da Universidade Federal de Juiz de Fora).

Keywords: Clinical Chemistry, Drugs, Nanotechnology
Application Code: Pharmaceutical
Methodology Code: Sensors
Graphene, the most attracting 2-D nanomaterials, possesses broad applications because of its excellent electronic, mechanical, optical and chemical properties. Particularly, by coupling metal nanoparticles onto the surface of graphene sheet, multifunctional nanocomposites have been proposed for metal-based graphene catalysis, surface-enhanced Raman spectroscopy, enhanced photothermal effect, etc.. Here, a general, environmental-friendly, one-pot method for the fabrication of reduced graphene oxide (RGO)/metal (oxide) (e.g. RGO/Au, RGO/Cu2O, and RGO/Ag) nanocomposites was developed using glucose as the stabilizer and reducing agent. The RGO/metal (oxide) nanocomposites were characterized using STEM, FE-SEM, EDS, UV-Vis absorption spectroscopy, XRD, FT-IR and Raman spectroscopy. The reducing agent, glucose, not only reduced the GO to form RGO, but also reduced the metal precursors to form metal (oxide) nanoparticles on the surface of RGO. Moreover, the RGO/metal (oxide) nanocomposites were stabilized by glucose on the surface of RGO. To demonstrate the utility of these materials in electrochemical sensing, the RGO/Au nanocomposite was used for the simultaneous detection of L-ascorbic acid (L-AA), dopamine (DA) and uric acid in natural matrices with detection limit of 0.1 mM, 1 µM and 5 µM, respectively.
The online combination of thermogravimetry with mass spectrometry (TG-MS) is widely used for evolved gas analysis (EGA). In recent years, also hyphenated systems featuring an additional gas chromatographic (GC) separation to form a TG-GC-MS were available. A newly developed system, which is presented in this study, expands the possibilities of common TG-GC-MS by implementing new features.

A novel modulator system and a fast-GC were developed. In combined use, it allows a short separation within a 30 s-temperature-programmed GC run. Advantages are the enhanced signal intensities, due to accumulation of the evolved gases in the modulator and the possibility to differentiate between isobaric and isomeric compounds on the basis of different retention time.

Furthermore, a time-of-flight (TOF) mass spectrometer with a maximum mass resolution of 5000 M/\(\Delta M\) was applied. It ensures high acquisition rates and enables the distinction of isobaric compounds, which is not possible with low resolution mass spectrometers.

Additionally, fast switching between hard electron ionization (EI) and soft single-photon ionization (SPI) is possible and allows quasi-simultaneous acquisition with both ionization techniques. This offers standard ionization energy mass spectra for library search in EI mode and mass spectra where the molecule ions are most abundant at the same time in SPI mode.

The new system was applied to characterize various petrochemical samples such as crude oils, oil shales, algae- and other bio-feedstock-based oils and fuels. The gained multi-dimensional data sets facilitate the determination of elemental compositions and give further insights into these highly complex matrices.
Evolved gas analysis using mass spectrometry (MS) is an established analytical technique in thermal analysis (TA) to further characterize the samples causing the mass loss. With current instruments, usually electron ionization (EI) is employed. As the kinetic energy of the electrons thereby is typically far beyond the ionization energies of the assayed samples, the interaction of electron and molecules effects fragmentation particularly of organic compounds, hampering the correlation of the ion signals to the gaseous compounds. This applies for complex mixtures in particular. Fragmentation can be reduced using so-called soft ionization techniques. In the course of the presented setup, single photon ionization (SPI) is employed.

For the instrumentation, a STA (Simultaneous Thermal Analysis) system has been coupled to a SPI-oaTOFMS (oaTOFMS: orthogonal acceleration time-of-flight mass spectrometry) system using a heated transfer capillary in order to detect semi volatile organic substances from the gas flow of the thermo balance with high temporal resolution.

In this work the thermo-analytical data and the SPI-MS information on the released organics is presented and discussed for various samples. Namely biomass (soft and hard wood), fossil fuel (crude oil and coal) as well as a complex polymer (ABS) are investigated. A first commercially available Setup will be presented and the general potential of hyphenating thermal analysis and soft photo ionization mass spectrometry (SPI-MS) for fundamental and applied research and material analysis is discussed.

Keywords: Mass Spectrometry, Other Hyphenated Techniques, Pyrolysis, Thermal Analysis
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Thermal Analysis
Polytetrafluoroethylene (PTFE) is, of course, well known as a non-stick coating for cookware. Because PTFE is very unreactive and provides a high chemical resistance, it is also used for medical applications and for industrial applications such as containers for reactive chemicals and for corrosion-resistant pipe liners. Also parts like bearings, bushings and gears, where sliding action is needed, are made from PTFE. In this paper, thermal characterization of a PTFE material was carried out using various thermal analysis and thermophysical properties testing techniques. Measurements were carried out between 170[degree]C and 700[degree]C (depending on the method). The thermal expansion and the density changes were determined using pushrod dilatometry (DIL). Dynamic mechanical analysis (DMA) was used to analyze the viscoelastic properties (storage and loss modulus). Thermal diffusivity was measured using the laser flash technique (LFA). Combining thermal diffusivity data with specific heat and density allows for calculation of the thermal conductivity of the polymer. The decomposition behavior was studied using simultaneous thermal analysis (STA). Evolved gases were analyzed by a mass spectrometer (QMS) and Fourier transform infrared spectroscopy (FTIR). In this paper, various thermophysical and thermomechanical properties were tested to get a better understanding of PTFE. The solid-solid transition could be identified by all the thermal analysis techniques that were used. Only dynamic mechanical analysis was able to detect transitions related to the amorphous phase.

Keywords: DSC, FTIR, Polymers & Plastics, Thermal Analysis
Application Code: Polymers and Plastics
Methodology Code: Thermal Analysis
Wood and wood pellets are important energy sources with a zero carbon dioxide balance. This means the carbon dioxide produced during combustion was originally captured by the plants. Instead of firing the wood in air, it could also be heated up in inert gas atmosphere to produce charcoal and also gaseous organic products. The pyrolysis of wood material was investigated by a thermo balance connected to a gas chromatograph with mass spectrometer (TGA-GC-MS). This instrument combination facilitates measuring the mass change of the substances versus temperature and time. The GC-MS allows the identification of gaseous products in real time (quasi-continuous), meaning direct correlation to actual temperature/time/mass. Alternatively, the gas products condensable at -50°C were collected in a cryo-trap and analyzed after the TGA measurement by high resolution GC-MS analysis. In this contribution, the principle of the TGA-GC-MS will be explained along with the measurement modes employed for these investigations. The TGA-GC-MS results will be shown and explained for selected wood samples.

Keywords: Biofuels, GC-MS, Pyrolysis, Thermal Analysis
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Thermal Analysis
Multidimensional Evolved Gas Analysis of Semiluxury Natural Foods by STA-GCxEI/SPI-TOF MS

After a single run, multidimensional information is available: The data set contains the thermal information for a chosen heating program from the STA and substantiates these with the correlating mass spectra. Detailed chemical information can be extracted from corresponding mass spectra. It is possible to operate MS in a fast switching modus between EI and SPI. The SPI mass spectra are dominated by the molecular ions of the evolved gases, due to the soft character of this ionization technique, while the molecular specific fragment pattern is available in the EI mass spectra. By CO2-modulation the evolved gases are trapped and remobilized in 30 s cycles to the column of the rapid GC. With regard to enduring repeatable complete rapid GC, runs a chromatographic separation of isobaric molecules is possible.

Quality control and process optimization in food industry are applications where STA-GCxEI/SPI-TOFMS can be employed. This is a challenge, as hundreds of molecules evolve from natural food products like tobacco, coffee or peanut, evolves simultaneously hundreds of molecules. The aim of this study was to show, that it is possible to simulate the roasting processes in the micro scale to track specific products or to gain specific chemical information of raw products.

Keywords: Food Identification, Natural Products, Thermal Analysis, Time of Flight MS
Application Code: Food Identification
Methodology Code: Thermal Analysis
It is well known that bile salts form micelles, but the precise understanding of the structure and dynamic behavior of the aggregation of bile salt monomers to form larger aggregates remains elusive. In this work, isothermal titration calorimetry (ITC) is used to characterize the thermodynamics of the sodium cholate bile salt micelle system, providing significant information about the stability and stoichiometry of this system. Furthermore, ITC experimentation should be well suited to measure the difference in micelle-binding energetics between the R- and S- enantiomers of 1,1’-bi-2-naphthol (BN) and 1,1-binaphthyl-2,2’-diylhydrogenphosphate (BNDHP), molecules that are known to bind to cholate micelles with differing affinity. Chiral resolution appears to be enthalpic controlled: these experiments should provide the first direct measurement of thermodynamics of stereo selective guest solubilization of binaphthyl substrates by bile micelles. Our early data suggest that the presence of BNDHP in high concentrations (2.0 mM) facilitates the micellization process and documents a significant difference in the binding energies between the two enantiomers of BNDHP. Interestingly, the temperature dependence of the demicellization enthalpy ($H_{demic}$) yields a positive change in heat capacity of demicellization ($C_{pdemic}$), suggesting that the dissociation of the aggregates of sodium cholate in water leads to the exposure of additional hydrophobic surface area. In all, ITC data that systematically investigates that cholate micelle system, as well as cholate binding of BN and BNDHP, will be presented.

Keywords: Bioanalytical, Characterization, Chiral, Thermal Analysis
Application Code: Bioanalytical
Methodology Code: Thermal Analysis