A new era of quantitative biology enabled by mass spectrometry based proteomic technologies has arrived. We can now define the content, relative abundance, modification states and interaction partners of proteins in a dynamic and temporal manner on a near-global basis in organelles, whole cells and clinical samples, providing information of unprecedented detail. At the Broad Institute we are employing these technologies in a wide array of studies including delineating the genetic underpinnings of mitochondrial disorders, connecting cancer genotype to molecular phenotype, unraveling the basis of the innate-immune response, identifying the mechanism of action of drug-like molecules and to discover and verify protein biomarkers of disease. A representative set of project vignettes will be presented to convey a sense of the breadth and depth of application of modern proteomics to biology and medicine.
Solid phase microextraction (SPME) has been discovered and developed at University of Waterloo more than 20 years ago. First clinical studies were conducted in Toronto General Hospital/University Health Network. This analytical method offers major breakthrough in field of extraction technologies, however its applications in clinical medicine are still limited. It does not require sample preparation therefore it allows direct extraction from any biological tissue or body fluid including blood. SPME principle relies on use of a novel biocompatible fiber or blade, which can be inserted into tissue or blood stream (intravascular cannula) and eliminates the need for drawing blood samples and quenching (stopping metabolic process). One fiber is capable of absorbing thousands of metabolites and medications. These unique features make SPME a perfect tool to study patient’s response to treatment, measure drugs concentration and study individual patient’s metabolic profile-metabolome.

The aim of this presentation is to discuss briefly these new applications of SPME, which potentially can lead to introduction of SPME to clinical practice. Specifically this presentation will focus on 4 possible uses.

1. Describe the role of SPME in measurement of drug concentration and subsequent pharmaco-modeling of medication commonly used in acute care medicine.
2. Use of SPME-based analytical platforms as a point-of-care tool allowing rapid detection of metabolites and drug levels in order to facilitate clinical decisions
3. Use of SPME–based analytical to describe metabolic “snap shot” of individual patients and their response to therapy and intervention (metabolomics). Describe the use of this platform as a first step towards individualized medicine.
4. Discuss potential, future applications aiming at identification of potential biomarkers serving as descriptors of organ’s function and dysfunction.

Keywords: Biomedical, Drugs, Metabolomics, Metabonomics
Application Code: Clinical/Toxicology
Methodology Code: Liquid Chromatography/Mass Spectrometry
Genetic variants influencing the transcriptome have been studied extensively. However, the impact of the genetic factors on protein expression and the proteome is largely unexplored, partly due to lack of suitable high-throughput quantitative methods. Here we present a unique set of identifications of genetic variants affecting the human plasma proteome achieved by combining label-free high-resolution LC-MS with genome-wide SNP data. We quantified 1,056 tryptic peptides representing 163 proteins in the plasma of 1,060 individuals from two population-based cohorts. The abundance level of one-fifth (19%) of the peptides was found to be heritable, with heritability 0.08-0.43. The levels of 60 peptides from 25 proteins were influenced by cis-acting SNPs. We identified and replicated individual cis-acting SNPs influencing 11 peptides from 5 individual proteins. These SNPs represent both regulatory SNPs and non-synonymous changes defining well-studied disease alleles such as the e4 allele of APOE, which has been shown to increase risk of Alzheimer’s disease. In this study, all statistical analyses were performed on the peptide measurements rather than aggregate or derived protein abundances. The peptides directly represent the MS measurements and better capture the protein heterogeneity in the populations. The results show that label-free LC-MS is a viable alternative to SRM, especially for the few hundred most abundant proteins. The composition of the proteome play an important role in the etiology, diagnosis, and treatment of a number of diseases, and a better understanding of the genetic influences on the proteome is important for evaluating potential biomarkers and therapeutic agents for common diseases[1].

Early diagnosis of neurological disorders is important for the treatment and management of neurological diseases. In this presentation, a high-performance isotope labeling liquid chromatography mass spectrometry (LC-MS) technique with demonstrated advantages of improved metabolome coverage and specificity for quantitative metabolome profiling of biofluids will be described. The applications of this technique for the discovery of specific metabolite biomarkers of several neurological disorders including mild cognitive impairment (MCI), Alzheimer's disease (AD) and autistic disorder (autism) will be presented. Potential applications of these biomarkers in clinical diagnosis of these disorders will be discussed.

Keywords: Clinical Chemistry, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry, Metabolomics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Mass Spectrometry
Sample preparation is frequently a bottleneck of bioanalysis. One of the technologies introduced to preclinical and clinical studies recently was solid phase microextraction (SPME). SPME offers many formats, which makes the method suitable for variety of applications. The overview of therapeutic drug monitoring, pharmacokinetics as well as targeted and untargeted metabolomics will be presented. This will include in vivo sampling with no sample withdrawal, extracted biofluid spot analysis and thin film microextraction. The importance of high throughput and automation in the view of clinical laboratory requirement is also fulfilled by SPME and the examples of larger scale drug screening will be shown. A few examples of in vivo tissue analysis during liver transplantation as the alternative to standard approach will be demonstrated. Finally, the future perspective of coupling SPME with mass spectrometers as rapid diagnostic tool for intensive care and surgery units will be discussed.
Recruiting and Onboarding New Staff

The people are the greatest asset of any organization. The people’s effort, expertise, and intellectual contributions are key to the organization’s success. Since people are critical to the outcome, it is a significant responsibility for leaders to effectively recruit, on-board, and retain the right people for their organization. This presentation will focus on our approach to recruiting and onboarding new staff. We will discuss our method of developing the job description, seeking candidates, and evaluating candidates. Once the right candidate is chosen, we will then discuss our process for bringing them up to speed as new employees.

Keywords: Lab Management, Laboratory
Application Code: Laboratory Management
Methodology Code: Other (Specify)
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**Abstract Text**

While core (shared) laboratories are becoming standard on university campuses and national research sites, their technical oversight requires a unique skillset that must be taken into consideration when recruiting support staff. From the employee’s perspective the mix of responsibilities of a shared lab – process development, user training, SPC, safety enforcement - appear to resemble neither the traditional “industrial R&D” mix of activities nor the “academic R&D” mix of PI-oriented activities. These shared labs demand technical competence, but also aspects of customer service traditionally associated with sales or retail occupations. Identifying individuals with these skills is only the start of their career support. There must be an on-going system to measure and reinforce them through a performance review process and an organization culture that values the full scope of staff activity. To create a context where an employee can take pride in their daily contributions, the enterprise leadership team needs to articulate the metrics of organizational success to employee, In this presentation the skill set for shared lab technical support will be described, along with strategies for recruiting individuals who will thrive in such an environment. Behavioral interviewing will be described as a tool to examine the “soft” skills of a candidate. The career path will be further described through the management activities of goal setting, performance appraisal, and career advancement. Dashboards will be presented as a tool for communicating organization progresses in measureable areas.

**Keywords:** Lab Management  
**Application Code:** General Interest  
**Methodology Code:** Other (Specify)
Characterization technology is recognized as a core competency within the Technology and Innovation Organization of SABIC’s Innovative Plastics Business. The Global Analytical Technology organization has developed this core competency over many years through a structure based on analytical disciplines. We refer to the groups that comprise this structure as “functional teams”. These teams represent communities of practice and are divided into two major areas: chemical and physical characterization. In chemical characterization the teams include vibrational spectroscopy, chromatography and separations, elemental and inorganic analysis, and NMR spectroscopy. For physical characterization, the teams include rheology and thermal analysis, mechanical properties and fracture mechanics, and microscopy and microanalysis. While not all analytical sub-disciplines are represented by these teams, the scope of competencies represents the vast majority of the solutions we provide. A key element of the teams’ charters is to develop technical "roadmaps". Components of the roadmaps include (1) developing competencies by executing development programs, interacting with external organizations, and best practice sharing, (2) building capability with respect to laboratory instrumentation and infrastructure, and (3) utilizing capacity for problem solving on a global basis. The functional teams provide an opportunity for grass-roots, technical strategy development, and have proven effective in attracting, engaging and retaining key talent. Examples of the processes used to engage individual contributors and managers will be shared.
Hiring and staff development strategies are important for maintaining the high performance of the internal analytical testing lab at Afton Chemical, a petroleum additives company located in Richmond, Virginia. In the past, the analytical group was used to screen entry level employees for positions in other areas of R&D, but increased demand for quality and throughput in analytical results has required a robust and skilled team in the analytical lab with a strong customer focus. Lab organization and culture will be described along with the steps used in hiring, training, and developing technicians to succeed individually and as part of the team.
Public service through science is a career option providing meaningful work and a reasonable living; there is satisfaction knowing that everyday you are helping your community and protecting the environment. Public agencies operate under civil service rules, intended to assure fairness in hiring; civil service rules also create situations that could be viewed as bureaucratic or rigid. However, understanding the rules clearly one can reach the right candidates and rules can become objective tools for employee development. This presentation explores how public agencies can effectively recruit quality laboratory professionals, develop them to be partners for the utility operation and retain them through meaningful work.

Utility laboratories play a supporting role rather than lead the organization, even though operations rely on the laboratory data to make decisions. The measure of a successful laboratory is the extent of help and support it provides to the organization. The formula for a successful and a fulfilling career in a utility laboratory is to engage broadly to understand utility operations, process control and regulatory drivers and developing the skills to translate and apply laboratory data to fine tune operations.

The successful recruitment of employees with desirable qualifications and characteristics starts with the laboratory management - knowing exactly what is needed for a particular organization. It is rare that one finds a candidate with all the desired experience, knowledge, and skills. The best option could be to find a candidate who can grow to be the ideal employee. With this option also comes the time intensive training, coaching, and assessment of the employee and the honest feedback that helps growth. If it is indeed a good match, the retention will follow naturally; if it is not a match, the laboratory management has the responsibility to act on it early.

Keywords: Lab Management, Laboratory, Process Control
Application Code: Environmental
Methodology Code: Education/Teaching
Cylindrical nanostructures derived from self-organized block copolymers have attracted considerable attention as chemical separation and sensing media because of their uniform diameters, tunable sizes and controllable chemical properties. For the last several years, our group has successfully demonstrated the chemical sensing capabilities of these structures using electrochemical and scanning probe techniques. However, it is often challenging to align such nanostructures along one direction over a millimeter scale. In addition, technical difficulties often arise when these materials are to be incorporated into confined spaces such as within microchannels. Efficient methods to align cylindrical domains within confined spaces are required for the future development of analytical devices based on these materials. This presentation will introduce our recent attempts to control the alignment of cylindrical domains in poly(styrene)-poly(ethylene oxide) block copolymer (PS-b-PEO) films formed within a micron-scale cavity formed between two glass substrates. The cylindrical PEO domains could be aligned along the direction of the penetration of 1,4-dioxane vapor and in the flow direction of a fairly concentrated PS-b-PEO solution. The extent of domain alignment was assessed by tracking the motion of individual sulforhodamine B molecules that preferentially partitioned into the PEO domains. Such single molecule tracking data provided a simple and quantitative means for assessing not only the degree of domain alignment, but also domain diameter and the diffusion behavior of individual probe molecules within the domains.

This work was funded by the Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences of the U.S. Department of Energy (DE-FG02-12ER16095).

Keywords: Imaging, Lab-on-a-Chip/Microfluidics, Nanotechnology, Polymers & Plastics
Application Code: Nanotechnology
Methodology Code: Microscopy
Ion transfer electrochemistry at liquid-liquid interfaces can be employed for the successful detection and sensing of chemical and biochemical species that do not exhibit useful redox electrochemistry. For example, the detection of bioactive molecules such as neurotransmitters and drug substances can be achieved based on transfer of the ionised molecules from the aqueous phase to an immiscible organic phase. Similarly, larger molecules such as proteins, polysaccharides and DNA can be detected, often by an adsorption/desorption approach that involves electrolyte ion transfer rather than transfer of the analyte species. By miniaturisation of these interfaces to the nanoscale, enhanced mass transport fluxes can be achieved to bring about better analytical performances. The formation of micro and nanoscale liquid-liquid interfaces has been achieved by use of appropriately-sized pipettes in recent decades. However, we have sought to use porous membranes placed at the interface in order to pattern the interface into an array of microscale or nanoscale interfaces.

This presentation will focus on the preparation and characterisation of arrays of nanoscale liquid-liquid interfaces formed within the pores of nanoporous membranes. The nanopores can be formed in various ways, including by use of electron beam lithography and reactive ion etching, or by direct-writing using focused ion beam milling. With such nanointerface arrays, we find enhanced diffusional fluxes leading to higher current densities, and with a concomitant increase in sensitivity as interface size is decreased. The analytical opportunities are explored by study of model ion transfer reactions, such as those of alkylammonium cations, and also by study of neurotransmitter and drug molecule transfers.

**Keywords:** Membrane, Nanotechnology, Sensors, Voltammetry

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
Block polymers can adopt various ordered morphologies with compositional heterogeneities on the nanometer length scale. Selective removal of one block from such structures leads to nanoporous materials that hold tremendous promise in various patterning, separations, and templating applications. Methods for the preparation of mechanically robust nanoporous materials with controlled structures from multicomponent block polymers containing reactive functional groups will be discussed.
Selective ion exclusion from charged nanopores in polymeric membranes allows separation of ions with different charges or mobilities. This presentation will initially examine pressure-driven transport of dissolved ions through membrane nanopores modified by adsorption of polyelectrolytes. For nominal 30 nm pores modified with a single layer of poly(styrene sulfonate) (PSS), bromide/sulfate selectivities are ~3.4 with sulfate rejections around 85% due to selective electrostatic exclusion of the divalent anion from the negatively charged pore. Related membranes containing an adsorbed PSS/protonated poly(allylamine) (PAH) bilayer are positively charged and exhibit average K⁺/Mg²⁺ selectivities >10 at 8 mM ionic strength, and Mg²⁺ rejections are >97.5% at ionic strengths <5 mM. Rejection and selectivity should increase as the pore size decreases and surface charge increases. Simultaneous modeling of K⁺ and Mg²⁺ rejections using the non-linearized Poisson-Boltzmann equation gives an average modified pore diameter of 8.4 ± 2.1 nm, which does not vary significantly with ionic strength. This diameter is smaller than that calculated from hydraulic permeabilities and estimated pore densities, suggesting that narrow regions near the pore entrance control ion transport. In addition to simple electrostatic exclusion, streaming potentials lead to differing rejections of bromide and acetate in PSS/PAH-modified pores, and of Li⁺ and Cs⁺ in PSS-modified pores. For these cases, electrical migration of ions toward the feed solution results in higher rejection of the more mobile ion. Current works aims at using applied potentials, thicker membranes, and highly uniform pore diameters to separate ions with similar mobilities.
This presentation will describe the fabrication and use of elastomeric nanochannels with size-tunable cross-sectional dimensions. In this nanofabrication procedure, a poly(dimethylsiloxane) (PDMS) slab with microchannel features are bonded to a thin PDMS film using plasma oxidation. When the resulting PDMS microchannel device is stretched in a direction parallel to that of the microchannels, an array of tunneling cracks are formed. While cracking typically occurs randomly, with the combined use of stress focusing features (SFF) and control of the degree of stretch to an extent that matches the spacing of the SFF, crack-based nanostructures can be formed with precisely defined locations and spacing. These cracks serve as tunable nanochannels where the cross-sectional area can be sufficiently widened to allow the efficient loading of large-radius coiled DNA and chromatin into the channels. When the strain is subsequently relaxed, the channel cross-sectional dimensions decrease significantly. This narrowing linearizes and traps biopolymers in an extended state without the application of an electric field by the generation of a mixed shear and elongational flow as well as through the effects of nanoconfinement. This nanoscale squeezing technique enables stretching of lambda DNA to its full contour length in physiological buffers. Additionally, the system enables differentiation of stretchability of chromatin reconstituted with and without histone H1, as well as multi-color mapping of histone acetylation and methylation of single strands of native chromatin isolated from mammalian cells.

Keywords: Bioanalytical, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
The presentation will focus on lessons I learned during my eight year tenure at the NSF about Center research and its level of effectiveness in developing novel chemical measurement and imaging methods. Examples of successful research projects in instrument and method development will be shown as well as examples from my own research where Center collaborations would not have been particularly helpful. The objective is to show that research Centers, which are currently under pursued by analytical chemists who often prefer to work individually and independently, could promote faster development of new and novel analytical methods, and to provide some practical advice about the development and management of NSF research Centers.

Keywords: Instrumentation
Application Code: Other (Specify)
Methodology Code: Other (Specify)
# A Center Approach for Creating and Studying Real World Chemical Complexity in the Laboratory in the NSF Center for Aerosol Impacts on Climate and the Environment

This presentation will highlight the research, education, and outreach goals and activities of the NSF Center for Aerosol Impacts on Climate and the Environment (CAICE). This Phase II Center for Chemical Innovation focuses on tackling the grand challenge of elucidating the chemical complexity and reactivity of atmospheric aerosol particles. A major goal of this Center is to perform controlled laboratory studies to better understand how complex aerosol chemistry plays a role in impacting our atmosphere and climate. This presentation will describe how the Center brings together an interdisciplinary team composed of chemists, as well as marine biologists, and oceanographers to provide diverse perspectives and approaches for gaining fundamental chemical insights into this important problem. Highlights of the novel methods used to produce representative aerosols will be discussed, including a state-of-the-art ocean-atmosphere wave flume facility to produce atmospherically relevant sea spray and a portable MART tank that can be used in laboratory studies. New experimental tools for spatially-resolved chemical analysis are being developed to explore the complex, heterogeneous, and dynamic aspects of aerosol particles bridging a broad range of length and time scales. The theme of environmental measurements unifies the research, education, and outreach missions of the Center. Graduate students are trained in developing new analysis methods involving small, portable, and robust instruments that can be used to make measurements of complex multiphase components in clouds, snow, rainfall, fogs, seawater, and air; outreach efforts involve elementary school students building small particle and gas phase sensors that can be flown on home-built rockets. A summary of the Center efforts, identified gaps and needs in measurement technologies, and future challenges will be presented.

**Keywords:** Aerosols/Particulates, Environmental Analysis, Environmental/Air, Instrumentation

**Application Code:** Environmental

**Methodology Code:** Mass Spectrometry
The motions of molecules manifest themselves on a sub-nanometer length scale and a femtosecond time scale. The processes that drive chemical reactivity, the making and breaking of a bond, take place on these ultrashort spatial scales and ultrafast time scales. Yet, current technologies are unable to capture molecules in the act with the appropriate spatial and time resolution. Consequently, much of what we know about ultrafast molecular motion and reactivity has come from theoretical and computational models without direct experimental validation. In the Center for Chemistry at the Space-Time Limit (CaSTL), a team of multiple research groups from different academic institutions has set out to push the state-of-the-art in instrumentation to achieve the time and space scales relevant to molecular reactivity. Efforts in the Center have let to substantial improvements in sensitivity for probing the ultrafast motions of individual molecules. By combining expertise from different disciplines including electron tunneling microscopy, atomic force microscopy, optical microscopy, ultrafast spectroscopy, molecular synthesis and theoretical modeling, researchers in the CaSTL Center have managed to record the first coherent and ultrafast movies of single molecules. In this presentation, we will highlight some of the key strategies and accomplishments by the CaSTL team.

Keywords: Raman, Surface Enhanced Raman, Ultra Fast Spectroscopy, Vibrational Spectroscopy
Application Code: Other (Specify)
Methodology Code: Molecular Spectroscopy
Center for the Physics of Living Cells at the University of Illinois, Urbana-Champaign is one of ten Physics Frontier Centers funded by the US National Science Foundation. We aim to develop path-breaking technologies based on single molecule and single cell analysis tools and computational microscopy collectively as a team of scientists from physics, chemistry, biology and bioengineering with interest in the inner workings of living cells. In this talk, I will describe two approaches.

(1) Single Molecule Pull-Down (SiMPull)
We developed a SiMPull assay that combines the principles of a conventional pull-down assay with single-molecule fluorescence microscopy and enables direct visualization of individual cellular protein complexes. SiMPull can reveal how many proteins and of which kinds are present in the in vivo complex and is widely applicable to various signalling proteins, and to endogenous protein complexes from animal tissue extracts. The pulled-down proteins are functional and are used, without further processing, for single-molecule biochemical studies.

(2) Single Molecular Tension Gauge:
Cell-cell and cell-matrix mechanical interactions through membrane receptors direct a wide range of cellular functions and orchestrate the development of multicellular organisms. To define the single molecular forces required to activate signaling through a ligand-receptor bond, we developed the tension gauge tether (TGT) approach in which the ligand is immobilized to a surface through a rupturable tether before receptor engagement. Using a range of tethers with tunable tension tolerances, we show that cells apply a universal peak tension of about 40 piconewtons (pN) to single integrin-ligand bonds during initial adhesion. We find that less than 12 pN is required to activate Notch receptors. TGT can also provide a defined molecular mechanical cue to regulate cellular functions.

Keywords: Fluorescence, Microscopy, Nucleic Acids, Proteomics
Application Code: Bioanalytical
Methodology Code: Microscopy
In 1994, the USA National Science Foundation funded a national user facility for ultrahigh resolution mass spectrometry. Over the years, the Facility has grown to six Ph.D. permanent staff, a technician, and a machinist. Postdocs and graduate students also collaborate with external users. Access is free, provided that (a) the problem cannot be solved with other mass analyzers, and (b) the results must be publishable. Instrumentation is upgraded continuously, not just at the onset of each new grant period. Current applications include proteomics (both bottom-up and top-down), complex organic mixture analysis (e.g., petroleomics, environmental samples), hydrogen/deuterium exchange to map contact surfaces in biomacromolecule complexes, and new cluster ions. We are preparing for the arrival of a 21 tesla horizontal superconducting magnet (highest magnetic field in the world for FT-ICR MS) in Fall, 2013. The talk will feature instrumentation developments and performance benchmarks as well as representative current applications, e.g., mass spectral peak capacity greater than 1,000,000, providing nonpareil resolution and identification of >100,000 elemental compositions from a single mass spectrum. Work supported by NSF DMR-11-57490 and NSF CHE-10-19193, BP/The Gulf of Mexico Research Initiative to the Deep-C Consortium, and the State of Florida.
I will discuss a new technique of generating user-controllable (time-varying and localizable), massively parallel stimuli on arrays of cells mediated by coalesced ensembles of labeled magnetic nanoparticles. At the core of this platform is a micromagnetic substrate composed of: i) electroplated soft magnetic (NixFey) elements, ii) a biocompatible, planarized resin layer, and iii) lithographically patterned cell-adhesion and cell-blocking layers to precisely align magnetic-nanoparticle-dosed cells upon controlling micro-magnets. Magnetizing the micro-magnetic elements with a robotically-controlled permanent magnet generates large, arrayed magnetic potential minima that can be used to ratchet particles across a substrate external to cells or localize particles with nanometer accuracy within cells. With forces applied by this platform we demonstrate coordinated responses in cellular behavior, including the PAK-dependent generation of active, leading-edge type filopodia, and significant (45 to 90 degree) biasing of the metaphase plate during cell mitosis. Large sample size and rapid sample generation also allow us to analyze cells at an unprecedented rate and obtain statistically robust data sets — a single sample can simultaneously stimulate thousands of cells for high statistical accuracy in measurements. The technique shows promise as a tool in both analytical and engineered approaches for cell analysis and control.

Keywords: Bioanalytical, Biomedical, Biotechnology, Nanotechnology
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Many immune-mediated diseases—infectious diseases like HIV and autoimmune diseases like multiple sclerosis—cause pathology in specific tissues, yet most of our knowledge about them has resulted from studying cells circulating in blood. Increasing evidence suggests, however, that the biology of diseases in affected tissues can vary substantially from that in the blood, and understanding these differences may be critical to improve patient care. The significant heterogeneities among cells resident in tissues necessitates characterizing such samples with single-cell resolution, but existing technologies routinely used by clinical immunologists (flow cytometry, ELISpot) require an excess of cells to use for analysis. Their inefficiencies can hinder the investigation of the human biology of diseases and treatments in tissues, because biopsies yield very few cells.

We have developed a modular analytical platform for characterizing functions, phenotypes, and in some instances, genotypes with single-cell resolution. This approach emphasizes the conservation of individual cells available from clinical samples by using dense arrays of subnanoliter wells (nanowells) that allow multiple measurements on each cell. The widespread adoption of this technology in the clinical research community, however, requires addressing certain critical technical elements identified by end-users. These elements include the throughput of unique clinical samples, breadth of data, data extraction and simple analysis/visualization, and cell recovery for clonal expansion or gene expression analysis.

In this talk, I will present advances related to the platform that improve the ability to functionally characterize rare cells such as mucosa-resident HIV-specific B cells and circulating tumor cells. Together, these advances are providing a path towards the use of this platform to support clinical studies in a range of human diseases, from autoimmunity and infectious diseases to cancer.
This presentation will describe a new, low-cost and high-throughput microfluidic device for the detection and molecular analysis of circulating tumor cells (CTCs). The device captures CTCs directly from unprocessed whole blood, provides on-chip cell labeling for CTC identification, and allows for facile cell-retrieval for further analysis. The device operation is based upon a size-selective cell separation technique, which was implemented by the weir-style physical barrier with a gap in the main fluidic channel; blood cells which are smaller than the gap height move straight through following a laminar flow, whereas larger cancer cells deviate from their original path and move along the physical barrier to be collected in a separate outlet. This new system is a versatile CTC analysis platform with many advantages. First, it supports extremely high throughput operation, since the use of weir structure effectively reduces fluidic resistance and enables flow-through separation. For example, we achieved >6000-fold CTC enrichment from whole blood at a high flow rate (10 mL/h). Second, the CTC-chip facilitates clear visual verification and enumeration of captured cells during/after operation. For this purpose, we have implemented microwell-shaped capturing structures on the physical barrier. When cancer cells introduced to the device, they were individually collected at each capture site at a single-cell resolution. Furthermore, the captured cells could be profiled in situ by introducing fluorescent antibodies. The chip thus assumes not only high detection sensitivity but also molecular specificity for CTC identification. Finally, the CTC-chip provides a facile way to retrieve captured CTCs. By reversing the flow direction, the cells can be dislodged from their capture sites and collected for further investigation (e.g., cell culture and genetic analysis).
### Abstract Text

We are investigating proteomics at the single cell level. While most techniques rely on the average signals from individual cells, our approach, based on digital ELISA and single molecule nucleic acid detection, has the ability to yield true single cell results. We are developing microfluidics systems to isolate and lyse single cells and then couple the lysate to ultra-sensitive single molecule protein and nucleic acid analysis assays. As a proof of concept, we are studying intracellular and membrane proteins involved in cancer pathways in different cancer cell lines.

**Keywords:** Biotechnology, Immunoassay, Lab-on-a-Chip/Microfluidics, Proteomics

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

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Microfluidics has emerged as a new paradigm in quantitative measurements of biomolecules and dynamic pathways across radically different spatiotemporal scales. In this presentation, I will share our research on developing ultra-sensitive microfluidic bioassays for quantitative analysis of cancer biomarkers. I will discuss the novel microfluidic platforms that we have developed, including bead-based digital PCR, actutable microwell-patterned ELISA chip, and integrated microfluidic microvesicle capture and analysis system. I will also demonstrate their applications to sensitive and quantitative biomedical analyses of genetic mutations, protein markers, and circulating exosomes derived from cancer patients.

Keywords: Immunoassay, Lab-on-a-Chip/Microfluidics, Nucleic Acids, Proteomics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
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**Abstract Text**

Like many other law enforcement organizations around the world, the US Federal Bureau of Investigation (FBI) has both a criminal and a counterterrorism mission. Due to the adversarial nature of the US criminal justice system, laboratory conclusions must be especially rigorous and follow a robust quality assurance system with many time-consuming verification steps. On the other hand, counterterrorism cases often require a much more immediate or "expedient" forensic response to ensure the safety and security of the public in the midst of evolving terrorism scenarios. Even many routine criminal cases could benefit from more rapid forensic analyses to produce actionable investigative leads. Our challenge is to balance the rigor required by the courtroom with the expediency demanded by ongoing threats. This presentation will describe the current composition of the FBI Laboratory, and examples of how those assets are used to address this challenge.

**Keywords:** Forensics, Portable Instruments

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Portable Instruments
This presentation reviews ambient ionization, emphasizing the spray-based methods of DESI and paper spray, and briefly covering their fundamentals. Then we discuss the interfacing of ambient ion sources with miniature mass spectrometers. We argue that the ambient ionization/miniature MS combination is essential for direct on-site mass spectrometry and show that the combination requires use of a discontinuous atmospheric pressure introduction (DAPI) system. The presentation includes numerous examples of ambient ionization mass spectrometry with drugs of abuse, toxic compounds, environmental contaminants and explosives. From these data performance characteristics of both commercial bench top and also miniature mass spectrometers are deduced for spray ionization. The importance of MS/MS data for qualitative and quantitative measurement of components of complex mixtures, including blood and other biofluids, is shown. Elaboration of ambient ionization to include instantaneous derivatization during ionization (reactive ionization) is readily achieved. The instrumentation discussed is relatively simple yet it has the potential to dramatically improve and speed up measurements made at the crime scene.

Keywords: Drug Discovery, Forensics
Application Code: Environmental
Methodology Code: Mass Spectrometry
Targeted NGS methods have potential to improve forensic biology, overcoming limitations imposed by capillary electrophoresis (CE systems) by enabling (1) analysis of multiple types of genetic polymorphisms in a single reaction, using a single workflow, (2) higher resolution genotyping of current markers, including detection of intra-STR sequence variation (3) recovery of the maximum amount of useful genomic information from degraded DNA samples, and (4) higher resolution mtDNA and mixture analysis.

Targeted sequencing allows the forensic analyst to broaden the number and types of markers and applications when compared to traditional CE. Data that span the genome can answer a wider range of questions in a single, targeted assay to relieve analysis limitations of challenging samples (complex mixtures and degraded DNA), flatten the analyst’s decision tree, reduce workflow complexity and minimize DNA consumption. In addition, new avenues of investigations become possible including molecular autopsy, RNA analysis of body fluid and tissue types, and non-human forensic studies.

Combining forensic STR markers with SNPs and other loci increases the overall discrimination power (especially for difficult samples), improves resolution of complex kinship cases and reveals phenotypic information about a perpetrator to assist with criminal investigations. Inherent accuracy of the method, the power of the MiSeq system, specific assays to meet current and future requirements, and dedicated forensic analysis software make routine forensic NGS applications possible.

Comprehensive Forensic Genomics has potential to assist forensic DNA experts to generate more results from more samples. Impact on public safety may be that more criminal cases are solved earlier in recidivist criminals’ careers, thus breaking the cycle of violence and curtailing victimization. This can produce substantial emotional, security, and economic benefits for individuals, communities, and taxpayers.

Keywords: Forensics, Genomics, Identification, Sample Preparation
Application Code: Other (Specify)
Methodology Code: Other (Specify)
In 1977, Lindley and Evett introduced modern Bayesian methods for forensic evidence interpretation to the forensic science community. This and related approaches have dominated the academic research related to the interpretation and presentation of forensic evidence. However, in recent years there have been number debates, in both academic and forensic communities, related to the applicability of these methods in the U.S. judicial system.

Broadly speaking, these methods require the explicit statement of two mutually exclusive, but non-exhaustive, models about how the evidence in a given situation has arisen; one usually corresponding to a defense model and one corresponding to a prosecution model. Once these models have been defined and the evidence collected, the forensic science expert is then required to present the evidence in a concise and transparent manner so that a decision maker can ultimately decide between the two proposed models of how the evidence has arisen.

The evidence that a forensic scientist has available to evaluate between the two models is generally composed of the following components: (1) a trace of unknown origin; (2) a sample from the specific source specified by the prosecution model and (3) a collection of samples from the alternative source population specified by the defense model. In certain applications, the choice of the alternative source population will be mandated by available databases or, in extreme situations, there will be no such samples available.

We will review some of the common sets of probability models and statistical approaches that forensic scientists use to characterize the support that the evidence provides for deciding between the prosecution and defense models. We will also discuss how the various sets of competing models can be addressed with the commonly available evidence. The general approach will be illustrated with an example of the trace element analysis of high purity copper evidence.

Keywords: Chemometrics, Data Analysis, Elemental Analysis, Trace Analysis
Application Code: Homeland Security/Forensics
Methodology Code: Chemometrics
I received my Ph.D. in environmental engineering and I am currently working for the US FDA as an analytical chemist. In my presentation, I will focus on how PittCon helps me to build up my career in the analytical field - from a short-course attendee to a short-course instructor and from an environmental scientist to an analytical chemist.

Keywords: Analysis, Education, GC-MS, Liquid Chromatography/Mass Spectroscopy
Application Code: High-Throughput Chemical Analysis
Methodology Code: Education/Teaching
Working in a global American 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 the following challenges:

1. Career goal
2. Innovation
3. Communication
4. Team work
5. Leadership

Keywords: Education
Application Code: Other (Specify)
Methodology Code: Other (Specify)
It has been said that whatever you can dream of, you can achieve. However, in reality it is not as easy as it sounds. In this presentation, the author, a passionate and dedicated scientist, shares her personal stories and experiences at work to demonstrate how she determines goals, builds confidence and overcomes the hurdles in her journey to turn her dreams into reality and get where she wants to be.

Keywords: Education
Application Code: Other (Specify)
Methodology Code: Education/Teaching
## CACA: How to be Successful in Your Career

### How to Face Challenges at Different Stages of Our Career – Lessons Learned

Every one of us faces challenges on our career development and growth in every stage of our career. What do we need to understand? What do we need to prepare for ourselves? How do we adjust ourselves to suite the work environment and the needs at different stages? What is the most important thing to do at the work place? There are many questions, particularly for those of us coming to US from different culture backgrounds. Some personal experiences and lessons learned will be shared. Hopefully, this may help stimulate some discussions and thinking.

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**Session Title**

CACA: How to be Successful in Your Career

**Abstract Title**

How to Face Challenges at Different Stages of Our Career – Lessons Learned

**Abstract Text**

Every one of us faces challenges on our career development and growth in every stage of our career. What do we need to understand? What do we need to prepare for ourselves? How do we adjust ourselves to suite the work environment and the needs at different stages? What is the most important thing to do at the work place? There are many questions, particularly for those of us coming to US from different culture backgrounds. Some personal experiences and lessons learned will be shared. Hopefully, this may help stimulate some discussions and thinking.
Infrared Spectroscopy (Well Beyond) the Diffraction Limit

Expanding Applications for AFM-Based Infrared Nanospectroscopy

Atomic force microscope-based infrared spectroscopy (AFM-IR) has been developed in recent years providing extremely high spatial resolution chemical characterization and imaging. The technique is based on the combination of a tunable infrared laser with an atomic force microscope that can locally map and measure thermal expansion of nanoscale regions of a sample resulting from the absorption of infrared radiation. Because the AFM probe tip can map the thermal expansion on very fine length scales, the AFM-IR technique provides a robust way to obtain interpretable IR absorption spectra at spatial resolution scales well below the diffraction limit. The technique also provides simultaneous and complementary mapping of mechanical properties and has been widely and successfully applied to applications in polymers and the life sciences.

Most previous AFM-IR measurements have been performed using total internal reflection illumination from below the sample, generally requiring samples to be prepared as thin sections transferred to an IR transparent prism. We have recently extended the AFM-IR technique to work in a "top side illumination" configuration. The top side illumination enables a much broader range of samples to be measured and can in some cases dramatically simplify sample preparation. Using top side illumination we have been able to measure samples including semiconductors, metal films, geological samples and others.

We have also implemented a "resonance enhanced" version of AFM-IR, demonstrated recently by Lu and Belkin. The resonance enhanced AFM-IR uses a tunable infrared source that is pulsed synchronously with a resonance of the AFM cantilever. Using this resonance enhanced AFM-IR, we have obtained high quality absorption spectra of samples as thin as 20 nm. We have developed techniques to automatically remove the probe absorption background resulting from the AFM cantilever being in the top-side illumination path.

Keywords: Infrared and Raman, Materials Characterization, Polymers & Plastics, 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 not only at VIS frequencies but also in the IR or THz spectral range.

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 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 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.

Equipping s-SNOM systems with cw light sources near-field imaging can be performed at time scales of 30-300s per image. Use of material-selective frequencies in the mid-IR spectral range can be exploited to fully characterize polymer blends or phase change materials with nanometer-scale domains. Quantification of free-carrier concentration and carrier mobility in doped semiconductor nanowires, analysis of Graphene nanostructures [3], or study phase propagation mechanisms in energy storage materials is achieved by amplitude- and phase-resolved infrared near-field imaging.


Keywords: FTIR, Materials Characterization, Microscopy, Microspectroscopy
Application Code: Nanotechnology
Methodology Code: Vibrational Spectroscopy
High-Resolution Mid-Infrared Micro-Spectroscopic Imaging with a Broadly Tunable Quantum Cascade Laser

Abstract Text
Conventional mid-infrared (mid-IR) Fourier transform infrared (FT-IR) spectroscopic imaging systems employ an incoherent globar source and achieve spectral specificity through interferometry. While this approach is suitable for many general applications, recent advancements in broadly tunable external cavity Quantum Cascade Lasers (QCL) offer new approaches to and new possibilities for mid-IR micro-spectroscopic imaging. We present a discrete frequency infrared (DFIR) microscope based on a QCL source and explore its utility for mid-IR imaging. Four QCL devices are multiplexed together to provide an overall system tuning range of up to 800 cm\(^{-1}\) spanning the fingerprint region, with sub-wavenumber spectral resolution. Spectral contrast is achieved by tuning the QCL to bands in the spectral region of interest. We demonstrate wide-field imaging employing a 128x128 pixel liquid nitrogen cooled mercury cadmium telluride (MCT) focal plane array (FPA) detector and high NA lenses with a sub-micron effective pixel size. The images presented will demonstrate high resolution, diffraction limited capabilities as well as several unique features due to coherence effects from the laser source. We will discuss the effects of this coherence and compare the spatial and spectral performance of our instrument to conventional mid-IR imaging instrumentation.

Figure 1: 1951 USAF resolution target patterned with SU8 photoresist on a BaF2 substrate. The absorbance spectrum is compared to FT-IR measurements of the same sample. These images, shown at select wavenumbers, are taken with a 0.56 NA BD-2 glass objective at 1.6 μm/pixel. 50 μm scale bar.

Keywords: FTIR
Application Code: Biomedical
Methodology Code: Other (Specify)
Infrared Spectroscopy (Well Beyond) the Diffraction Limit

Characterization of Materials Using AFM-Based Nanomechanical, Nanothermal, and Nanoscale Infrared Spectroscopy and Imaging

The enhanced physical and mechanical properties of polymer nanocomposites are likely intimately related to the individual component chemical interactions. Since these critical interactions occur on small spatial scales, enhanced nanoscale chemical characterization techniques will be needed to help understand them. Nanothermal analysis, nanomechanical spectroscopy combined with Lorentz contact resonance imaging, and infrared (IR) nanospectroscopy and imaging are newly emerging approaches that can provide valuable insights into the nanostructure of composites and biomaterials. Each of these techniques will be described and examples of their application presented. Understanding gained from the use of these new AFM-based nanoscale characterization tools should help to accelerate the development of polymer nanocomposites with superior properties.

Keywords: Infrared and Raman, Materials Characterization, Microspectroscopy, Nanotechnology

Application Code: Materials Science

Methodology Code: Molecular Spectroscopy
Localized surface plasmon resonances couple propagating light with nanoscale volumes of matter (hot-spots), enabling new applications in sensing and therapeutics. Surface-Enhanced Infrared Absorption (SEIRA) Spectroscopy exploits these hot-spots for sensitive chemical detection. Calculations predict large SEIRA enhancement factors for small hot-spots but the diffraction of IR light has so far prevented the experimental determination of SEIRA enhancements with nanoscale resolution.

Photo Thermal Induced Resonance (PTIR) combines the chemical specificity of IR spectroscopy with the lateral resolution of Atomic Force Microscopy (AFM). PTIR uses a tunable pulsed laser for sample illumination and an AFM tip in contact as a local detector. The absorption of a laser pulse results in sample heating, sample expansion, and mechanical excitation of the AFM cantilever, used as a “spatial filter” to extract the local chemical composition with nanoscale resolution. The local IR spectrum is obtained by plotting the tip deflection as a function of wavelength. Notably, the PTIR signal is proportional to the absorbed energy (not scattering) and the PTIR spectra are directly comparable with IR spectral libraries, enabling materials identification.

In this work, the PTIR technique is applied for the first time to image the dark plasmonic resonance of gold Asymmetric Split Ring Resonators (A-SRRs) in the mid-IR with nanoscale resolution. Additionally, the chemically-specific PTIR signal is used to map the local absorption enhancement of PMMA coated A-SRRs, revealing hot-spots in the resonators’ gaps with enhancement factors up to 30. We believe that Surface-Enhanced PTIR will facilitate the engineering and application of plasmonic nanomaterials.
Infrared Spectroscopy (Well Beyond) the Diffraction Limit
Structure and Morphology in Triaxial Electrospun Fibers

Electrospinning is a fiber forming technique that has recently experienced a dramatic increase in interest and activity. The production of fibers through this approach allows the control of polymer structure and morphology with fiber diameters as small as 50 nanometers. The chemical and mechanical properties of these nanofibers are often critical to the macroscopic performance of the fiber mat in applications as diverse as filtration and cell growth. There is a limited range over which mechanical properties can be varied for a given polymer fiber. However if a heterogeneous fiber structure can be created, then the possibilities for changing both chemical and mechanical properties becomes much broader. One approach to this involves creating a radially heterogeneous fiber structure through co-axial and triaxial electrospinning. Such an approach has been developed using concentric needles in a conventional electrospinning arrangement. Results for both co-axial and triaxial fibers will be discussed.

Keywords: Infrared and Raman, Nanotechnology
Application Code: Materials Science
Methodology Code: Vibrational Spectroscopy
Proton and electron transport in proteins have been studied with vibrational coherence spectroscopy and ultrafast kinetics. Green fluorescent protein (GFP) and cytochrome c (cyt c) are employed as model systems. Because of the inherent rigidity of the GFP beta-barrel structure, conformational disorder is minimal, which opens a window to observe the quantum aspects of proton transfer in proteins. A large and temperature dependent kinetic isotope effect (KIE) in GFP demonstrates that tunneling is taking place along the O-H---O elements of the GFP “proton wire”. An improved model for proton tunneling is developed, which formally includes the thermally accessible vibrational mode of the donor (D) and acceptor (A) oxygen atoms in the tunneling reaction coordinate. It is shown how the quantum mixing of the D-A mode with the much higher frequency O-H oscillator delocalizes the tunneling wavefunction and offers shorter tunnel pathlengths that control the underlying transport rates. Recent studies have also demonstrated how distortions along the “ruffling” mode of the heme group in cyt c can control the electron transport rates over two orders of magnitude. It is shown, generally, how low frequency (\(\frac{\kappa}{k_BT} \sim 300\text{K} \sim 200 \text{ cm}^{-1}\)) excitations associated with such distortions are able to extract energy from the thermal bath and utilize it for barrier crossing or tunneling. Moreover, these low frequency modes have the ability to mix with other delocalized low-frequency modes of the protein or with binding partners, offering a potential control mechanism.

Keywords: Method Development, Protein, Raman, Vibrational Spectroscopy
Application Code: Biomedical
Methodology Code: Vibrational Spectroscopy
We present a rigorous formulation of the task-specific information in a measurement. We apply this formulation to the analysis of optical imaging systems for target detection and parameter estimation tasks. Initial work on the design of information-optimal measurements will also be presented.
Abstract Text

The problem of inferring events of interest by fusing and exploiting data from multiple sensors has a wide variety of applications such as security of personnel both in combat as well as in everyday life. The inference tasks could consist of detecting an activity of interest or estimating some parameters, such as locations or tracks, which provide actionable intelligence and/or improved situational awareness. The sensors deployed in a given region of interest, in the most general setting, may consist of rather disparate and incommensurate modalities, e.g., audio, video, and chemical sensors. Even sensors of the same modality may exhibit differences in their sensing ability, due to differences during manufacturing, quality control or the duration and location of their deployment. In other words, with respect to the information content of the signals, sensors exhibit heterogeneity that can arise from a wide variety of causes. Another factor that influences the performance of such a multi-sensor system is the fact that the sensors observe different aspects of the same phenomenon, i.e., sensor observations are dependent. The nature of this dependence can be quite complex and nonlinear. In this talk, we will present some background on data fusion methodologies and present some recent results on fusion of heterogeneous and dependent data for enhanced situational awareness. Some sample applications will also be presented.

Keywords: Data Analysis, Informatics, Sensors, Statistical Data Analysis
Application Code: Homeland Security/Forensics
Methodology Code: Integrated Sensor Systems
To plan and execute efficiently, humans seem to have an intuition for how to select only the most important constraints to help simplify our problems. For space missions, we may ignore certain faults in favor of focusing on preparing for others. For cyber security, we may remove assets believed to be compromised in order to increase expected reliability.

In this talk, we present our work on incorporating this intuition into model-based planning and execution. In addition to allowing the user specify goals and deadlines, they can also specify the amount of risk they are willing to accept. Our executive balances their risk-tolerance with the uncertain timing and effects inherent to the problem to produce and execute a plan.

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Keywords: Automation, Computers
Application Code: Other (Specify)
Methodology Code: Computers, Modeling and Simulation
A generalized framework based on information structures is discussed. This enables combining information from multiple sources and deriving decision rules for optimal utility of resources. Combining orthogonal information is a natural corollary of this generalized approach. This generalized framework is agnostic of application and system components. It has merits to be applied to diverse mission objectives.
Orthogonal and Risk-Based Sensing Systems for Homeland Security Applications

Measurement Bounds for Sparse Signal Ensembles via Graphical Models

In compressive sensing, a small collection of linear projections of a sparse signal contains enough information to permit signal recovery. Distributed compressive sensing (DCS) extends this framework by defining ensemble sparsity models, allowing a correlated ensemble of sparse signals to be jointly recovered from a collection of separately acquired compressive measurements. In this paper, we introduce a framework for modeling sparse signal ensembles that quantifies the intra- and inter-signal dependencies within and among the signals. This framework is based on a novel bipartite graph representation that links the sparse signal coefficients with the measurements obtained for each signal. Using our framework, we provide fundamental bounds on the number of noiseless measurements that each sensor must collect to ensure that the signals are jointly recoverable. This is joint work with Michael Wakin, Dror Baron, Shriram Sarvotham, and Richard Baraniuk.

Keywords: Data Analysis, Sample & Data Management, Sample Preparation, Sampling

Application Code: General Interest

Methodology Code: Sampling and Sample Preparation
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<td>Automatic Detection of Unknown Explosive Materials</td>
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<td>Primary Author</td>
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**Abstract Text**

Terrorists are attempting to use so-called 'Home-Made Explosives' (HME's) to attack our transportation infrastructure. The difference between these HME's and traditional explosives, is that the list of ingredients and recipes used are unknown and vary significantly from batch to batch.

The main challenge facing Explosive Detection Systems (EDS), is to design automated threat detection algorithms capable of detecting all HME's without necessarily having access to sample explosives.

We will present a proven methodology for how to approach this problem using CT based EDS. The method is applicable to other modalities as well.

**Keywords:** Data Mining, Detection, Sensors, Statistical Data Analysis

**Application Code:** Homeland Security/Forensics

**Methodology Code:** X-ray Techniques
Abstract Text
In a previous paper (G&I 07-08/2013 Trace Analysis of HF Impurities), the safety, material selection, and sample condition considerations were explored for optimal impurity analysis in anhydrous hydrogen fluoride gas (AnHF). This study will expand on these ideas, and delve into the techniques used to maximize sensitivity and minimize detection limits when analyzing impurities in AnHF. Important areas for consideration are: instrument selection and configuration, gas cell/path length selection, proper sampling techniques, and calibration techniques. In addition to the above topics, this study will also determine the most common impurities found in AnHF from the major specialty gas manufacturers, and benchmark a CIC Photonics IRGAS system to determine the detection limits and sensitivity that can be achieved when measuring these impurities.

Keywords: FTIR, Gas, Monitoring, Process Monitoring
Application Code: Process Analytical Chemistry
Methodology Code: Other (Specify)
Analytical accuracy is critical to supply controlled parameters for specific use such as quality control procedure or demonstration of regulation compliance. The key issue is that classical validation methods do not consider the evaluation of impurities in complex matrix with multiple interferences. The objective of this presentation is to illustrate the use of the Accuracy Profile Method to define the appropriate interference studies necessary for validation of analytical methods such as infra red techniques. This methodology requires that during method development for a defined concentration range, additional validation is necessary using a wider range of the analyte concentrations. Experiments are undertaken to collect reproducible measurements under a variety of conditions. The idea is to vary one component in a typical mixture to represent different cases of specific use. Classical statistical parameters (standard deviation for accuracy, bias for trueness, variation coefficient) are estimated. An acceptance limit for uncertainties and tolerance probability (known proportion of future measurements) have to be defined following the requests of final application. Then, using experimental data obtained with high precision & accuracy calibration mixtures, the accuracy profile model allows to estimate the tolerance interval in order to accept or reject the investigated analytical method.

Keywords: FTIR, Validation
Application Code: Validation
Methodology Code: Data Analysis and Manipulation
The NIST Gas Metrology Group has developed a new suite of atmospheric sulfur hexafluoride Primary Standard Gas Mixtures (PSMs) that range from (5 to 10) pmol/mol (ppt) with < ± 0.20% (0.02 pmol/mol) relative uncertainties. Four levels of standards were made, 12 cylinders total. Each PSM cylinder was weighed on an automated balance between each fill step and all PSMs were analyzed on a GC. Impurities of pure gases were measured and included in all fill calculations to help ensure the lowest possible uncertainties. GC data and gravimetric concentrations were plotted to a general least squares program, GenLine. The average residual difference between the predicted values and the gravimetric values of the PSMs will be presented. This suite of primary standard gas mixtures will be used to certify two new NIST Standard Reference Materials (SRMs) 1720 Northern Hemisphere Air and 1720 Southern Hemisphere Air.
Driven by the global need for accurate and traceable zero gas standards, a European initiative has been working for the last three years on the development of a protocol for the certification of zero gas. The research focused on the determination of reactive gases impurities (e.g. nitrogen dioxides and sulfur oxide) in nitrogen and clean air as they, if present, may significantly alter results in air quality measurements. The presentation will show details of the developed procedure of certification and examples of the validation results.
Oxygen is a critical impurity in bulk and specialty gases for many applications, ranging from semiconductor fabrication to chemical manufacturing. Present sensing technologies typically rely on direct contact between oxygen molecules and the detection device, e.g. in zirconia sensors, electrochemical sensors, or catalytic converters. As a consequence, use of these techniques in challenging gas matrices, e.g. ammonia or corrosive gases such as HCl or chlorine, is impossible in many cases. In addition, relatively low initial costs can be offset over time by significant operating costs due to consumables, maintenance requirements, and the need for calibration gases. Tunable diode laser sensors operating around 760 nm offer a non-contact solution; however, the weak absorption strength of oxygen in this spectral region limits the sensitivity of this technique to ppm or percent levels. Continuous-wave cavity ring-down spectroscopy (CW-CRDS) overcomes this sensitivity limitation by employing a high-finesse optical cavity. In contrast to another approach employed by Tiger Optics which combines CW-CRDS with catalytic conversion, this new instrument utilizes direct spectroscopic detection via the oxygen A-band with no moving parts or active surfaces. While catalytic conversion CW-CRDS offers outstanding performance in inert gases, only the direct spectroscopic system is able to measure trace O$_2$ in almost any gas matrix, including hydrides, corrosives, and acid gases. In addition, the direct-O$_2$ CW-CRDS instrument offers a lower detection limit in the part-per-billion range, a compact footprint, no maintenance requirements, no consumables, and freedom from calibration gases. Measurements of trace oxygen in inert and specialty gases are presented.
Hydrogen elimination mass spectroscopy (HEMS) has the potential of simplifying the analysis and the quantification of impurities in hydrogen gas in a variety of applications. Enrichment factors (ratio of the initial moles of hydrogen in the sample to the moles of hydrogen remaining in the sample after hydrogen elimination) greater than $1 \times 10^8$, can readily be achieved. Removing most of the bulk hydrogen allows the impurities in the sample to be quantified in many different ways, from quantifying the quality of fuel-station grade hydrogen (contaminants ranging from high ppm to low ppb), analyzing sub-ppb impurities in ultra-pure hydrogen required in compound semiconductor applications or measuring the total maximum contamination in the hydrogen by simply measuring the total pressure in the sample after the hydrogen elimination.

The core of the HEMS is a chamber separated by a Pd-alloy membrane, which enables the removal of hydrogen from the gas sample while retaining all the impurities. In a typical sample, an enrichment factor of $1 \times 10^6$ to $1 \times 10^8$ is targeted. With the hydrogen greatly reduced the analysis can use a variety of analytical tools; currently, a quadrupole mass spectrometer is utilized.

In this presentation the work reported on focuses in measuring the linearity, zero drift, and repeatability of the instrument for both SAE-J2719 spec hydrogen and 9-9’s hydrogen supply gas. Using different processes and components, factors such as Methanation, coking of the carbon-based impurities, changes in the reactive species and adsorption of sulfur species during sampling and analysis are reported on.

Keywords: Fuels\Energy\Petrochemical, Mass Spectrometry, Specialty Gas Analysis
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Other (Specify)
Process Raman spectroscopy has found many applications in various processes in which the characterization or composition analysis of solids and liquids are required. Interestingly, it was the control and optimization of the crystal form of titanium dioxide, a solid powder that became one of the earliest successful on-line applications for modern Raman spectroscopy.

While the potential for gas-phase Raman spectroscopic measurements, within the laboratory, has been well established it is only recently with the introduction of gas-phase optimized Raman analytics by Kaiser Optical Systems that a field of in-situ gas-phase Raman spectroscopy has opened up. This presentation describes some key features of gas-phase Raman spectroscopy, key needs in making quantitative gas-phase Raman measurements, and includes several successful applications where Raman has replaced traditional gas-phase measurement modalities.
The demand for high purity ammonia (NH₃) has increased with the growth of the LED market. Monitoring impurities at the part per billion (ppb) level are now standard practice in the LED manufacturing facilities.

LED manufacturers demand high NH₃ purity to increase yield and "brightness". UHP ammonia can be delivered to or produced at the manufacturing site, purifiers are often used to ensure gas quality at or close to the tool, but on line monitoring is the only "real time" strategy to determine gas quality feeding the tool chamber (in or out). Monitoring low level moisture (H₂O) is currently the standard for ammonia, but other impurities may be faster/valuable indicators of gas quality and purifier or system failure.

Commercial process gas chromatographs (GCs) equipped with reducing compound photometer (RCP), flame ionization detector (FID), and, pulsed discharge helium ionization detectors (PDHID) have been widely used in wafer manufacturing industry to monitor ppb level impurities in ultra high purity (UHP) bulk gases for decades. Methods have been developed for monitoring impurities at the 5 ppb level in ammonia with these GCs. These methods utilize a suite of GCs, with standard configuration to measure hydrogen (H₂), carbon monoxide (CO), carbon dioxide (CO₂), total hydrocarbon (THC) - as methane + non-methane hydrocarbons, argon/oxygen (Ar/O₂*), and Nitrogen (N₂) in ammonia.

* Note: Oxygen is not measured discrete from argon and cannot be determined reliably at 5 ppb.
Two approaches currently exist within the literature for the detection of two-dimensional (2D) peaks in 2D chromatograms, the watershed algorithm and the two-step algorithm. We have previously shown that the two-step algorithm detects additional peaks compared to the watershed algorithm. However, previous investigations of the two-step approach have only been applied to a single wavelength. We therefore propose two modifications to the two-step approach. First, we suggest the generation a base peak chromatogram by selecting the largest absorbance at each time point. Second, we suggest the implementation a spectroscopic constraint in addition to the normal degree of overlap constraint during the merging process. We examined the impact of these two modifications on the peak detection of 2D chromatograms of maize seed extracts. We excluded any 2D peaks with a height less than 5 mAU. As a point of reference, we selected 220 nm as the representative 2D chromatogram. We detected 62 2D peaks at this reference wavelength. When performing detection on the base peak chromatogram, we detected 72 2D peaks (an improvement of 15 %). Further, when the background was corrected prior to the formation of the base peak chromatogram, we detected 87 2D peaks (an improvement of 42 %). The inclusion of the spectroscopic merging constraint further resulted in the detection of 87 – 123 2D peaks (an improvement of 42 to 98 %) depending on how stringent the criterion was applied. We therefore recommend the inclusion of a spectroscopic dimension during 2D peak detection.

Keywords: Data Analysis, HPLC
Application Code: Other (Specify)
Methodology Code: Data Analysis and Manipulation
Determining sites of metabolism and rapidly sharing that biotransformation information, allows quicker structure design to dodge liabilities in drug discovery. Metabolite structures are the key to biotransformation schemes, invariably prepared with effort and multiple software packages despite their ubiquitous importance. While LC/MS data interpretation alone is becoming more automated, prediction-based approaches are of increasing interest in discovery, [DD Stranz, et al. Toxicol Mech Methods. 2008, 18, 243; V Zelesky, et al. Bioanalysis. 2013, 5, 1165]. Our innovative software platform with a new module for metabolite prediction and user-assisted targeted LC/MS data extraction facilitates building a biotransformation map. Then structures together with live data are easy disseminated and colleagues may view, edit or search information as compounds progress so this software innovates at a crucial aspect of metabolite identification research: live metabolism summaries made simple.

A new module expedites the process by in silico metabolite prediction wherein phase I metabolites of a user-selected compound are obtained based on which of its atoms are the most likely targets for one of the five key human cytochrome P450 isoforms: CYP3A4, CYP2D6, CYP2C9, CYP2C19, CYP1A2. A combination of modeling strategies yield a composite probability and reliability for each site of modification and applying reactions to those yields the discrete structures, which are transferred automatically to the software interface and selecting update database creates a biotransformation map of all potential structures. A user initiates automated mass spectrometry data processing, with chromatogram extraction and peak detection, and then further refinement of which peaks correspond to which metabolites. Where ambiguities remain, a user can collapse isomers to a Markush structure before the map and supporting data are updated in the knowledge management platform.

Keywords: Drug Discovery, Liquid Chromatography/Mass Spectroscopy, Scientific Data Management, Software
Application Code: Drug Discovery
Methodology Code: Data Analysis and Manipulation
Clinical staff in Intensive Care Units are often extremely busy dealing with critically ill patients and so are running in a 'crisis mode'. Typically, data collected from bedside monitors has been collected at low resolution (~1 Hz) and typically stored at very low resolution (4 samples/hour) and only offered limited information into the changing condition of these critically ill patients. Clear information, available on the ICU, that could provide insight into the status of traumatically injured patients would be extremely valuable.

In the case of neurotrauma, using high resolution (>100Hz) data to relate chemical measurements (brain oxygen levels, PbtO2) with physiological measurements (Intracranial Pressure, ICP) could offer valuable feedback about the process of moment to moment cerebral autoregulation, or the vital control of blood flow through the brain. High resolution waveforms provide additional layers of information that are not visible in low resolution data.

Here, we will describe the techniques used to extract high resolution waveforms of brain oxygen levels and Intracranial Pressure from bedside monitors that are associated with systemic changes in cerebral autoregulation. Additionally, we will demonstrate the benefit of correlating these two items. In summary, this method enables data acquisition technology to provide feedback on a cerebral process whose basis is not currently well known.

Abstract Text

Keywords: Biomedical, Data Analysis, Monitoring, Neurochemistry
Application Code: Biomedical
Methodology Code: Data Analysis and Manipulation
### Abstract Text

In this talk, we report progress towards a usable direct “brain-instrument interface”. The vast majority of modern chemical instrumentation has at least a portion of its components that are electronic. Over the last hundred years, the electronic components have changed form and function drastically. What may have originally been a knife switch and d'Arsonval meter became multipole switches, knobs, pots, dials and then lights. Then as computers became available, keypads, keyboards and CRTs entered the arena and with A/D and DACs dominated the instrument composition. More recently, advances in operating systems have added a mouse or similar cursor movement method to the “operator-instrument interface”. As solid state fabrication improved, faster, more powerful and smaller electronic instrumentation have appeared. Room-sized became desktop. Desktop became portable. Portable became handheld and now much chemical instrumentation is small enough to be placed in situations that physical interaction between operator and instrument is difficult: space, sub-surface terrestrial and marine or sequestered within organisms or behind radiation shields. The operator-instrument interface has still required sufficient physical control that it is beyond the capabilities of those with even some modest physical limitations. To address demands placed on the operator-instrument interface, we have adapted commercially available headsets usable for recording EEGs from an operator's brain and used the signals to control several pieces of modern chemical instrumentation. Many instruments are controlled via programs written by the manufacturer. We have used the headsets, headset manufacturer's signal interpretation software, and instrument manufacturer's software to operate several instruments: UV-Vis, IR, NMR, GCMS, LCMS and microwave plasma OES. We will report results from instruments employing Windows, Linux and OSx operating systems.

### Keywords

- Computers, Instrumentation, Laboratory

### Application Code

- General Interest

### Methodology Code

- Computers, Modeling and Simulation
A simple approach for preparing standard mixtures of volatile and semi-volatile organic compounds was developed. When placed in a closed container, standard mixture components partition between a polymeric material such as poly(dimethylsiloxane) (PDMS) and headspace to provide constant vapor concentrations. The granular form of heat-conditioned PDMS provides rapid equilibration with the headspace vapor and serves as a standard reservoir. With a 7.4 mL device containing 2.0 g of PDMS, sampling for more than 114 times during a test period of 43 days, resulted in chromatographic peak area %RSD values below 4.5% for a variety of compounds. This device was designed to be solventless, quantitative, reproducible, environmentally-friendly, and robust for routine evaluation and calibration of gas chromatography–mass spectrometry (GC-MS) systems. The performance of both GC and MS components of the instrument can be monitored with vapors generated using this device. Other applications including GC column validation, internal standard generation, and on-site generation of calibration curves have also been explored. Financial support from Torion Technologies and Smiths Detection is gratefully acknowledged.
The extraction mechanism of solid phase microextraction (SPME) fiber coatings has been an area of increased interest due to the device's simplicity, high throughput nature, and high selectivity. Knowledge of the extraction mechanism for a SPME sorbent coating (i.e. adsorption or partitioning) can ultimately lead to a more detailed understanding of its analytical performance. Additionally, as there are distinct advantages and limitations to each type of extraction mechanism in SPME, it is beneficial to gain insight to these properties when selecting a suitable sorbent coating. An investigation into the mechanism of extraction for polymeric ionic liquid (PIL)-based SPME sorbent coatings will be discussed. Four PIL-based sorbent coatings, namely, a poly(vinylbenzylhexadecylimidazolium bis[(trifluoromethyl)sulfonyl] imide) PIL produced through 2,2'-azo-bis(isobutyronitrile) (AIBN) initiated free-radical polymerization, a UV-initiated poly(1-vinyl-3-hexylimidazolium) chloride PIL, and two crosslinked PILs containing the same IL monomers copolymerized with dicationic IL crosslinkers, were investigated to determine whether adsorption or partitioning was the dominate extraction mechanism. Using an experimental model which explores the competition behavior between select analytes with respect to the sorbent coating, the primary extraction mechanism for all PILs was determined. The details of the experimental method, along with the results and their interpretation, will be discussed. This work was funded by the National Science Foundation, Division of Chemistry (Analytical and Surface Chemistry Program, CHE-0748612).
The Importance of a Dry Extract for Alternative Chromatographic Carrier Gas Use

In recent years a shortage of helium has led to reconsideration of carrier gases for chromatographic use. Several solutions have been offered to mitigate or solve the problem including paying higher costs, helium conservation in the lab and the use of alternative gases for gas chromatography (GC) and GC/MS. Hydrogen is an alternative with promise to provide sensitivity and performance. However, it can also react with methylene chloride, commonly used for environmental extracts, in the presence of water to form HCl. This can cause corrosion in the inlet and exacerbate the problem of sample degradation. Although lowering the injector temperature can reduce the problem, other issues may arise. The ideal solution would be to provide a sample that is dryer to reduce the possibility of this problem.

This paper will compare the residual water content in an extract treated with sodium sulfate vs. that treated by passing through a membrane to separate the water. The implications for contamination, speed, recoveries and instrument performance will be discussed.

Keywords: Environmental, Membrane, Solvent, Water
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Ionic liquids (ILs) are a class of molten organic salts composed solely of ions while exhibiting lower melting points (< 100 °C), negligible vapor pressure, high thermal stability and electrical conductivity. The physico-chemical properties of ILs can be tuned for the specific task by varying the structure of the cation/anion of the IL. Magnetic ionic liquids (MILs) are a special class of ILs which are paramagnetic in nature and respond to a strong magnetic field. MILs can be designed to incorporate either a magnetoactive cation (ferrocenium salts) or anion (tetrachloroferrate salts). MILs can be applied as extraction solvents to preconcentrate the analytes from the aqueous matrix. After performing the extraction, they can be separated from the sample matrix by exposing them to the strong magnetic field. However, most of the magnetoactive anions, when combined with imidazolium cations, produce MILs that are highly hydrophilic in nature. Therefore, the homogenous solution of MIL in water cannot be separated even when exposed to a strong magnetic field (1 Tesla). Tuning and modification to the MIL structure will be discussed in an effort to enhance the extraction process and at the same time allowing quick separation of the MIL from the sample matrix. We will primarily focus on the different strategies employed to synthesize and characterize the hydrophobic MILs while preserving the important characteristics of ILs that make them highly useful in sample preparation.
Pulsed Amperometric Detection (PAD) is one of the most widely used detection methods for carbohydrate analysis in High Performance Anion Exchange Chromatography (HPAE). Carbohydrates with at least one electroactive functional group can be detected with high sensitivity after their separation on a chromatographic column using an alkaline eluent. A quadruple PAD waveform was introduced for the electrocatalytic oxidative detection of carbohydrates in our lab over ten years ago. It has improved the long term detection stability thanks to an effective cleaning step which is included in it. The cleaning step includes reductive and oxidation potential pulses to remove adsorbed species and products of electrode reaction from the electrode surface during each waveform period. Consequently, it significantly minimizes the possibility of electrode fouling compared to a single constant potential. However, the long-term detection performance of quadruple waveform for trehalose, fructose, and levoglucosan has been found less than optimal in some cases. As we have found out, this has not been caused by a poor performance of the cleaning step. Achieving an improved performance required an optimization of the detection potential. We have modified the magnitude and duration of the potential pulse designed for electrocatalytic oxidative detection at a gold working electrode under alkaline conditions. The modified waveform was evaluated for the determination of trehalose, fructose, and levoglucosan. An enhanced detection stability was experimentally confirmed.

Keywords: Bioanalytical, Electrochemistry, HPLC Detection, Ion Chromatography
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Hydrogen sulfide (H$_2$S), along with carbon monoxide (CO) and nitric oxide (NO), is a gaseous signaling molecule known as a gasotransmitter. These molecules help regulate ion channels within the body. Amperometric sensors have shown great promise in NO detection, however relatively recent identification of CO and H$_2$S as gasotransmitters has drawn queries regarding sensor selectivity as well as the interaction between the different gasotransmitters. There is a particular desire to selectively detect H$_2$S in vivo due to physiological significance in the central nervous and cardiovascular systems. There is clearly a need to independently detect H$_2$S in the presence of NO and CO in order to fully understand both its individual physiological roles and its interactions with NO and CO.

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 electrodeposited dicyano-ferrizporphyrin as such a material, which may be able to be incorporated into a future sensor design. However, certain limitations, most notably decreased sensitivity in either the presence of the interfering gases or consecutive trials, must be overcome prior to implementing this material into a practical sensor. This presentation will report on our recent work in understanding and advancing this electrocatalytic material toward practical applications.

This work is sponsored through an award from Research Corporation for Science Advancement and the National Science Foundation.
Kidney transplantation is the treatment of choice for patients with end-stage kidney disease, however, there is a severe shortage of suitable donor organs. One solution to this problem is to expand the donor pool to include the use of marginal donor organs, such as those from Non-Heart-Beating-Donors. Organs from such donors are currently under-utilised as they may have been subjected to a greater degree of ischemic injury prior to recovery, which could impair function in both the short and long-term. Therefore it is vital to be able to reliably assess the viability of these organs, in order to reduce the discard rate and increase the number of successful transplants.

We are developing an automated microfluidic analysis system that uses sensors to quantify key biomolecular markers of tissue health. This system allows us to continuously monitor the metabolic state of two organs simultaneously, in real-time. The kidney is sampled using microdialysis and the resulting dialysate stream is monitored for changes in glucose and lactate. The system consists of LabSmith devices that allow control of the dialysate streams, as well as addition of enzymes for amperometric detection. The system is programmed to calibrate automatically at regular intervals during the long periods of monitoring.

Preliminary results will be presented from both porcine and human kidneys and pancreases during hypothermic and normothermic machine perfusion and will be compared to those obtained using our existing rsMD system [1,2].

We thank the NIHR Biomedical Research Centre of Imperial College London for funding.

References:


Generator-collector assemblies, consisting of recessed disks array (generator) with a gold layer (collector) deposited over the top plane insulator, have been used for the selective steady state detection of dopamine (DA) in the presence of ascorbic acid (AA) [1]. The recessed disks potential was set onto the DA wave plateau, whereas the top plane was biased on the AA irreversible wave plateau before the onset of the DA oxidation wave. The plane electrode thus scavenged AA, so that the array of recessed disk electrodes monitored mostly the dopamine. The DA wave being nearly reversible chemically, its signal was further amplified by its redox cycling between disk and plane electrodes. This sensing principle was proven to be very effective and demonstrated also for other usual analyte/interfering species pairs and, surprisingly, operated as well when the top plane electrode was left floating [1]. Simulation approach has been elaborated to predict the behavior of the system [2] and was validated by new experimental sets. This revealed the crucial role of the plane electrode area which screens access to the recessed disks (i.e. acts as a diffusional Faraday cage) and simultaneously amplifies the analyte signal through positive feedback amplification even when it was left floating by acting in a bipolar mode. Simulations helped to assess quantitatively these two effects for different geometries, suggesting optimized operation modes for increasing the sensor sensitivity without altering significantly its selectivity [2].

This work was supported by ANR Chaire d’Excellence “MicroNanoChem” (ANR-10-CHEX-012-01), CNRS-ENS-UPMC (UMR 8640 “PASTEUR” and LIA “XiamENS”), the French Ministry of Research and by the Natural Science Foundation of Fujian Province of China (2012J01054).


Keywords: Bioanalytical, Electrochemistry, Microelectrode, Voltammetry
We have exploited unique interactions of gold, metal oxide and carbon nanostructures with biofilms formed by Pseudomonas aeruginosa, Staphylococcus aureus and Yersinia enterocolitica bacteria on glassy carbon electrodes. Regardless the general tendency of gold and silver nanoparticles (suspended in aqueous solutions) to minimize formation of biofilms on solid surfaces, once immobilized within porous conducting polymer (e.g. poly(3,4-ethylenedioxythiophene) or PEDOT) layers, they tended to facilitate growth of robust and mature bacterial biofilms on their surfaces. Independent diagnostic experiments showed that biofilms grown by the following bacteria, P. aeruginosa ATCC 9027, Y. enterocolitica Ye9, Y. enterocolitica AR4, L. monocytogenes 10403S and L. monocytogenes 1115, on inert carbon substrates exhibited by themselves electrocatalytic properties towards oxygen and hydrogen peroxide reductions in neutral media. The processes were found to be further enhanced by introduction of certain metallic (Au, Ag, Pd) and bi-metallic (Au-Pt) nanoparticles both unsupported and supported on such inert metal oxide nanostructures as TiO2 and ZrO2. Coexistence of the above components leads to synergistic effect that is evident from some positive shift of the oxygen reduction voltammetric potentials and significant increase of voltammetric currents. Further, the proposed hybrid films exhibited relatively higher activities towards reduction of hydrogen peroxide. Comparative measurements were performed aiming at better understanding of electrocatalytic efficiencies of various systems including those utilizing metal nanoparticles (e.g. Au-Pt), conventional enzymes (e.g. laccase), molecular systems (e.g. metalloporphyrins) in the presence and absence of selected bacterial biofilms.

This work was supported by National Science Center (NCN), Poland under Maestro project.

| Keywords: | Bioanalytical, Electrochemistry, Electrodes, Materials Characterization |
| Application Code: | Bioanalytical |
| Methodology Code: | Electrochemistry |
The formation of lipid-laden macrophage foam cells is the hallmark of the early stage of atherosclerosis. Some methods including biochemical assays and neutral lipid staining have been used for the detection of lipid in cells. However, none of these methods can fulfill the label-free and real-time quantitative assessment of the lipid content in living macrophages, particularly for its kinetic performance in the presence of drugs. Here we used coherent anti-Stokes Raman scattering (CARS) microscopy and further developed an automated image analysis method based on the maximum entropy thresholding (MET) to quantitatively measure the lipid content in living macrophages. The validation of CARS image analysis was demonstrated by the strong correlation (R-squared > 0.9) with the biochemical assay. Using this method, we monitored the processes of lipid accumulation and hydrolysis in living macrophages. We further characterized the lipid hydrolysis process in the absence and presence of a lipid hydrolysis inhibitor (diethylumbelliferyl phosphate, DEUP), determining the kinetic parameters such as the inhibition constant, Ki. Our work demonstrates that the automated quantitative analysis method is useful for the studies of cellular lipid metabolism, evaluating the potency of lipid synthesis or hydrolysis inhibitors, and has potential for high-throughput screening of therapeutic agents related to atherosclerosis and other lipid-associated disorders.

Keywords: Biomedical, Microscopy, Quantitative, Raman
Application Code: Bioanalytical
Methodology Code: Microscopy
## Abstract Text

Messenger RNA is, by its nature, transient, beginning with transcription and ending with degradation, but with a period of processing and transport in between. As such, the spatiotemporal dynamics of specific mRNA molecules are difficult to image and detect inside living cells, and this has been a significant challenge for the chemical and biomedical communities. To solve this problem, we have developed a targeted, self-delivered, and photocontrolled aptamer-based molecular beacon (MB) for intra-cellular mRNA analysis. An internalizing aptamer connected via a double-stranded DNA structure is used as a carrier probe (CP) for cell-specific delivery of the MB designed to signal target mRNA. A light activation, or photocaging, strategy was employed by inserting two photolabile groups in the CP sequence, enabling control over the MB’s intracellular function. After being guided to their target cells via specific binding of aptamer AS1411 to nucleolin on the cell membrane, light illumination releases the MB for mRNA monitoring. Two signals from the dye-labeled aptamer monitor cellular entry and function of the CP/MB probe through mRNA hybridization. First, probe entry is monitored by Cy5 signal through FRET from Cy3. Second, probe detection of mRNA by MB is monitored by Cy3 signal through direct excitation. The Cy5 signals also serve as an internal reference for ratiometric analysis. Consequently, the MB is able to perform live-cell mRNA imaging with precise spatiotemporal control, while the CP acts as both tracer for intracellular distribution of the MB and internal reference for mRNA ratiometric detection.

### Keywords
- Bioanalytical, Imaging, Microspectroscopy, Nucleic Acids

### Application Code
- Bioanalytical

### Methodology Code
- Microscopy
Islets of Langerhans are responsible for whole body glucose homeostasis. Reactive oxygen species (ROS), are detrimental to cells at high concentrations, but are hypothesized to be signaling molecules in islets which have low antioxidant capacity. The objective of this project is to determine the roles that glucose metabolism and changes in intracellular 
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\text{[Ca}^{2+}]_{i}\n\]
play in ROS production in islets of Langerhans.

The protocol to measure ROS consisted of incubating batches of 3-6 islets at 37 degree Celsius in varying glucose concentrations. Islets were then loaded with the ROS-sensitive fluorescent dye, H$_2$DCF-DA, and the fluorescence was measured using an epifluorescence microscope. The experimental protocol was optimized to minimize photobleaching, and consisted of using a neutral density filter with a minimum acquisition time combined with high sensitivity CCD detection. It was found that 3 mM glucose induced 1.5-fold higher fluorescence signal than 20 mM glucose. To determine the roles of 
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\text{[Ca}^{2+}]_{i}\n\]
and glucose metabolism on ROS levels, diazoxide was added which clamps open K$^{+}$ATP channels reducing 
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without affecting glucose metabolism. The presence of diazoxide increased H$_2$DCF-DA fluorescence at all glucose concentrations. Addition of 30 mM K$^{+}$ increased 
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\text{[Ca}^{2+}]_{i}\n\]
reducing the fluorescence by 15%.

With the developed protocol, we successfully determined that increases in 
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\text{[Ca}^{2+}]_{i}\n\]
decrease ROS levels, potentially to be used in intracellular signaling cascades. This report will focus on the analytical optimization of the measurement scheme as well as the novel biological findings.

**Keywords:** Bioanalytical, Fluorescence, Imaging, Microscopy

**Application Code:** Bioanalytical

**Methodology Code:** Microscopy
Biofilms are complex multicellular communities embedded in a polymeric matrix and attached to a surface that are responsible for problems ranging from the spread of infection to biofouling in fluidic systems. In this study Surface Plasmon Resonance imaging (SPRi) is coupled with microfluidics to monitor bacterial movement, growth, and biofilm formation along the entire surface of the channel in real time with sub-10 micrometer resolution. The imaging technology allows us to observe rapid physical changes at the channel surface, which is needed when studying motile cells in flow systems, over a 1 cm square area.

The cells and biofilm are detected by shining collimated light onto a gold sensor surface that forms the bottom wall of the microfluidic channel. The incident angle of the light is tuned such that it is absorbed and transformed into a plasmon when the surface is clean. When a biomolecule attaches to the surface, the plasmon cannot form and the light is reflected onto a charge-coupled-device (CCD).

To validate this new approach, we monitored the activity of two different strains of Pseudomonas aeruginosa (PA14), a model organism for biofilm and pathogenesis studies: Wild Type, which produces biofilm, and PelA mutant, which grows without producing biofilm. The two strains were injected into separate microchannels. The channels were imaged using SPRi and differences in biofouling rates between the two channels were easily observed. We plan to employ this technique to monitor the physiological response of bacteria exposed to dynamic flow conditions and chemical gradients.

**Keywords:** Biofuels, Biosensors, Contamination, Industrial Hygiene

**Application Code:** Industrial Hygiene

**Methodology Code:** Surface Analysis/Imaging
Simultaneously obtaining spatial and temporal resolution of the chemistry in live biological systems such as tissue sections is a difficult analytical problem. However, better understanding of a biological systems chemical environment with high spatiotemporal resolution can help scientists further understand the driving forces behind many normal and pathological biological phenomena. Current optical imaging techniques are typically limited to a few analytes and rely on fluorescent molecules. The objective of this research project is to develop electrochemical instrumentation that can monitor the chemistry occurring across an intact, living tissue slice with high spatial and temporal resolution.

The approach to be discussed couples microfluidics and high density electrode arrays fabricated on CMOS microchips. As designed, the electrode array contains over 8,000 individually addressable working electrodes and operates on the stage of an optical microscope allowing the chemical imaging to be correlated with traditional optical imaging. Microfluidic channels are used to generate controlled chemical gradients over the electrode arrays for initial proof of concept testing as well as the ability to provide fresh media to the tissue slice. Nitric oxide, dopamine, norepinephrine, and epinephrine have been analyzed with this system thus far. The development, challenges, and accomplishments of these high-density electrode arrays will be discussed.

Keywords: Bioanalytical, Biosensors, Lab-on-a-Chip/Microfluidics, Microelectrode
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Biofilms are of great interest owing to their role in bacterial infections and resistance to antibacterial agents. Much effort has focused on inhibition of biofilm formation with little effort directed toward the initial adhesion of individual bacteria to surfaces. To study these early stages of adhesion, we are using three-layer microfluidic devices fabricated with integrated pumps and valves, which automate the steps of cell culture, synchronization, and analysis. *Caulobacter crescentus* is a model bacterium for studying adhesion because of its dimorphic life cycle. As the bacterium transitions from a motile swarmer cell to a sessile stalked cell, the swarmer cell sheds its flagellum, forms an adhesive holdfast, and develops a stalk. This holdfast formation can be initiated by either cellular development or contact with a surface. Developmental holdfast formation occurs naturally after ~20-30 min, whereas holdfast formation initiated by surface contact occurs more rapidly (e.g., less than 1 min). To study the role of the flagellum in surface contact initiated holdfast synthesis, we compare the adhesion of wild-type cells and flagellum motor mutant cells (*motB*) in the presence and absence of a crowding agent, which promotes holdfast formation without surface contact. Synchronized populations are pumped to an analysis channel where holdfast formation events are tracked by fluorescence microscopy. A fluorescently labeled lectin binds with N-acetylglucosamine in the holdfast to observe holdfast formation on-chip. Individual cells are tracked from the swarmer stage through holdfast production to determine the rates of holdfast formation.

**Keywords:** Bioanalytical, Fluorescence, Lab-on-a-Chip/Microfluidics, Microscopy

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Bioanalytical Microfluidics

### Synchronization of Islets of Langerhans Using a Microfluidic Feedback System

In vivo insulin is secreted in a pulsatile manner with a period of 4 – 10 minutes which are essential for effective glucose uptake, but these pulses are perturbed in diabetic patients. One hypothesis for how one million islets of Langerhans synchronize to produce these pulses is through a feedback loop involving insulin-sensitive tissues. Because all islets sense blood glucose levels, a high glucose concentration in plasma will stimulate secretion of insulin from all islets. Increased blood insulin levels will then initiate a lowering of glucose levels. This reduction in glucose is sensed by the entire islet population, reducing insulin secretion, causing the glucose level to rise and initiating the cycle again.

In this study, an automated PDMS-glass hybrid microfluidic device was used to test this hypothesis to determine if a population of 6 – 10 islets could be synchronized. Intraislet calcium ([Ca2+]i) was imaged using Fura-2 fluorescence, converted to insulin, and used in a mathematical model of insulin-sensitive glucose uptake. The new glucose value was then delivered automatically to islets using a microfluidic perfusion system.

When the automated feedback was ON, glucose oscillations developed and the individual islets became synchronized and produced population oscillations of ~6 min. When a low value of insulin sensitivity was used in the model, it resulted in no glucose oscillations and hence no synchronization among the islets. These results support the hypothesis that synchronization of islets of Langerhans can be obtained using a glucose feedback response from insulin-sensitive tissues.

### Abstract Text

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### Keywords:

Automation, Bioanalytical, Fluorescence, Lab-on-a-Chip/Microfluidics

### Application Code:

Bioanalytical

### Methodology Code:

Microfluidics/Lab-on-a-Chip
Currently, the most prominent method used in microfluidics to render biocompatibility to aqueous-in-oil droplets is to synthetically produce a triblock copolymer surfactant composed of perfluoropolyether and polyether blocks. The surfactant products (EA surfactant, KryJeffa, etc.) sustain highly biocompatible droplet surfaces while maintaining the heat stability of the starting material (Krytox 157 FSH). However, production of these surfactants requires expertise in synthetic organic chemistry, creating a barrier to widespread adoption in the field.

We describe a simple alternative to synthetic modification of surfactants to impart biocompatibility. We have observed that aqueous-in-oil droplet surfaces can be made biocompatible and heat stable by merely exploiting binding interactions between polyetherdiamine additives in the aqueous phase and carboxylated perfluorocarbon surfactants (Krytox) in the oil phase. Droplets formed under these conditions are shown to possess biocompatible surfaces capable of supporting picoliter-scale protein assays, droplet polymerase chain reaction (PCR), and droplet DNA amplification with isothermal recombinase polymerase amplification (RPA). The binding interaction was confirmed with FT-IR spectroscopy, NMR spectroscopy, ESI-MS, and fluorescence microscopy. Overall, our results suggest that by simply introducing commercially-available, polyetherdiamine additives to the aqueous phase, researchers can avoid synthetic methods in generating biocompatible droplet surfaces capable of supporting DNA and protein analysis at the sub-nanoliter scale. This finding should allow biological applications in picoliter droplets to be more widely accessible.

Additionally, we have developed synthetic methods to promote biomolecule recruitment to the oil/water interface of these droplets. This way, we have begun exploring the possibility of performing quantitative bioassays at droplet surfaces.
Integrated electrodes in fluidic devices have been a powerful form of detection for nearly two decades, but electrode reusability and precision of placement in the device after modification or polishing is not trivial. Here, we describe a removable electrode system integrated into a fluidic device that was fabricated using a 3-dimensional (3D) printer. Electrodes were fabricated by embedding gold and silver wire, both 0.5 mm in diameter, inside a commercially available polyether ether ketone (PEEK) fitting with commercially available C-7 epoxy. After polishing and coating the silver wire with AgCl to form a quasi-reference electrode, the electrode set was coated with 5% w/w Nafion. The Nafion coating was further optimized for maximum oxygen diffusion through the coating to the electrodes. The 3D device, with a channel 3 mm in width and 0.5 mm in height, was designed to house a threaded fitting, allowing for integration of the electrode system directly above the channel. Oxygen standards, made with sodium sulfite, were pumped through the device, and a calibration curve was generated from standards ranging from 0 - 8 ppm. The bare electrodes were characterized outside of the 3D device using ferricyanide standards. Using cyclic voltammetry, sensitivity and limit of detection for ferricyanide using the electrode system with no surface coating was 0.3 mA/mM and 56.6 nM ± 9.7 nM, respectively, and the diffusion coefficient for ferricyanide was determined to be 2.98 x 10^{-6} cm²/s ± 4.08 x 10^{-7} cm²/s. Evaluation of these electrodes in the printed device will be presented.

Keywords: Bioanalytical, Biological Samples, Electrodes, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Bioanalytical Microfluidics

Development of a Microfluidic Device Assay for Isoforms of a Serum Protein Cancer Biomarker Using a Novel Antibody

Serum is replete with biomarkers that are mixed with high-abundance proteins such as albumin and immunoglobulins. Immunoassays that involve serum require very specific antibodies to avoid false positives. We are currently developing a microfluidic-based assay for detecting a cancer linked serum protein biomarker using a proprietary and high-specificity monoclonal antibody. This protein biomarker has promise in staging cancer and in prognostication that can help determine the correct course of therapy. Current immunoassays cannot detect the different isoforms of proteins. Therefore, it would be useful to determine the quantities of different isoforms of the target protein as a function of disease states in a fast and inexpensive assay. The monoclonal antibody was conjugated with FITC, and the antigen-antibody complexes were monitored by microchip electrophoresis laser-induced fluorescence. Initial results indicate various antigen-antibody complexes were observed in cancer (case) serum samples while complexes decreased or were absent in normal serum samples. The microchip electrophoresis was performed within 5 minutes. We are currently investigating improved sample preparation protocols and optimizing separation conditions. Development of these immunoassays for complex biological fluids using microchip electrophoresis may contribute to routine diagnostic tests and improved health outcomes.

Keywords: Bioanalytical, Biological Samples, Electrophoresis, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Hypoxia or oxygen deficiency is one of the critical features of human solid tumors. Hypoxia is the result of insufficient and defective vasculature present in highly proliferative tumor masses. Oxygen deficiency in these tumor masses can often lead to resistance to conventional cancer therapies. Therefore, it is important to study the efficacy of cancer drugs in both normal as well as hypoxic conditions. Microfluidics provides an excellent platform for the study of drug response towards cancer cells. Hypoxic condition is attained using the hypoxic chamber and oxygen level monitoring is performed using oxygen sensitive dyes. Multilayer soft lithography is used to fabricate a multi-region microfluidic device capable of culturing 8 different cell-lines at the same time at low-shear environment. Cell seeding is straightforward using vacuum actuation and apoptosis assays can be performed using variety of probes. Mitochondrial dye MitoTracker Deep Red is used to assay the loss of membrane potential in the cells treated with anti-cancer drug at normoxic and hypoxic conditions. Comparison between various cell-lines is performed based on their response towards the drugs at hypoxic condition. Independent control of oxygen and the nutrient supply in the device helps in creating a tumor microenvironment for tumor cells to study the drug response.
A complete analysis setup addressing key limitations of both benchtop and portable systems is highly desirable. We have developed microfluidic devices with visual inspection readout of a target’s concentration from microliter volumes of solution flowed into a microchannel. Microchannels are constructed within polydimethylsiloxane (PDMS), and the surfaces are coated with receptors. Capillary flow of target solution into the channel crosslinks the top and bottom surfaces, which constricts the channel and stops flow. The capillary flow distance of the target solution in the channel before flow stops enables the target’s concentration to be measured without detection instrumentation. Solution capillary flow distances scaled linearly with the negative logarithm of target concentration for streptavidin down below 1 ng/mL [1]. We have also characterized the mechanism of target specific constriction in these devices. Presently, we are evaluating microfluidic systems for sequence-specific DNA quantitation and measuring biomarkers via immunoaffinity interactions. Because of its easy readout and portability, our system has great potential for use in point-of-care diagnostics.

Reference

Keywords: Bioanalytical, Biosensors, Portable Instruments, Quantitative
Application Code: Bioanalytical
Methodology Code: Chemical Methods
Clinical microdialysis is gaining widespread attention in the treatment of brain injury. The injured brain is highly prone to waves of cortical spreading depolarization (CSD). Rapid-sampling microdialysis (rsMD) in patients with brain injury shows that CSDs deplete brain glucose. This occurs despite hyperemia, as blood flow to the brain is unable to meet the huge glucose demand imposed as the tissue repolarizes after the CSD. In the injured brain, recurring CSDs set up an especially dangerous scenario by driving glucose levels progressively lower, eventually leading to secondary injury, i.e. expansion of the core brain lesion that might occur long after the primary injury. CSDs are highly correlated with poor patient outcomes, including death, vegetative state, and severe disability 6 months after injury. To fully realize the potential of clinical microdialysis, significant improvements are needed. A major challenge is to solve the problem of insertion injury created by the microdialysis probe. Increasing evidence suggested that the insertion injury, while not dangerous to the patient because the probe is very small, compromises the reliable detection of CSD-induced glucose depletion.

Our objective was to monitor the effects of probe insertion in real-time using 2-photon microscopy in a transgenic mouse expressing GFP in microglia. Sulforhodamine101 was injected IV as an optical vascular label. Second, the probe tracks were examined by immunohistochemistry and fluorescence microscopy. Pharmacological agents were also introduced into the perfusate. The goal of this work was to identify key variables that affect the extent of insertion injury and acute tissue responses.

Keywords: Fluorescence, Medical, Neurochemistry
Application Code: Biomedical
Methodology Code: Microscopy
Nanoparticles with special physical and chemical properties have been extensively used as drug or gene delivery vehicles into biological cells for targeted therapy or biomedical imaging applications. So far, how viral particles take advantage of cellular endocytic machinery that leads to efficient invasion is still not well understood. Understanding of interactions between cell membrane and viral-simulant particles will greatly promote the advancement in these targeted delivery studies. We recently found that strongly negatively charged particles can be trapped near also negatively charged glass surface. Such trapping will play an important role in accumulating nanoparticles near the interface, resulting in an enhanced targeted binding and internalization efficiency of the particles. In this study, we study modified gold nanoparticles diffusing near synthetic membranes on glass surface. This work provides new insights for the better understanding of particle-surface interactions and biological processes on living cell membrane such as endocytosis and bio-recognition. Rational design of drug delivery vehicles can be developed based on this study. 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.
We have created novel near-infrared-emitting nanoscale metal-organic frameworks (nanoMOFs) that can be incorporated into living cells for near-infrared (NIR) imaging. We present both bulk and nano-Yb-PVDC-3, a new MOF based on a phenylenevinylene dicarboxylate (PVDC) sensitizer-ligand and Yb[\textsuperscript{3+}] NIR-emitting lanthanide cations. This material has been characterized using single crystal X-ray diffraction, powder X-ray diffraction (PXRD), and scanning electron microscopy (SEM); excitation and emission spectra, quantum yields, and luminescence lifetimes have been obtained under different solvents. Its stability in various media has been assessed through SEM, PXRD, and fluorescence measurements. We demonstrate that it is stable in certain specific biological media, does not photobleach, and has an IC\textsubscript{50} of 100 [\textmu g/mL which is sufficient to allow live cell imaging. The internalization of nano-Yb-PVDC-3 by HeLa and NIH 3T3 cells is verified with confocal microscopy and inductively coupled plasma (ICP) measurements. Despite its relatively low quantum yield, nano-Yb-PVDC-3 emits a sufficient number of photons per unit volume to serve as a NIR-emitting reporter for imaging living cells with efficient discrimination between the nanoMOF emission signal and the cellular autofluorescence arising from biological material. This work represents one of the first demonstrations of the possibility of using NIR lanthanide emission for biological imaging applications in living cells with single photon excitation. This research was funded by La Ligue contre la Cancer.
For its widely application in industrial and personal care products, zinc oxide nanoparticles (ZnO NPs) have drawn considerable concerns regarding their potential environmental and public health risks. Whereas the underlying physicochemical and molecular mechanisms of ZnO NPs cytotoxicity remains unrevealed. We’ve showed the irradiation induced free radicals generation in ZnO NPs and thus enhanced cytotoxicity in our previous study, which would be closely affected by -potentials, dispersant ion strength, and dissolved oxygen. In this study, a thorough investigation of the toxicity mechanism in normal skin cell line was carried out for a comprehensive understanding of interaction of ZnO NPs with bio-systems. In particular, the cytotoxic effects were carefully and quantitatively evaluated and compared between free zinc ions and ZnO NPs. We found out that oxidative stress and cell loss was partially rescued by anti-oxidant N-acetyl cysteine (NAC) in free zinc ions treated groups, whereas the rescue efficiency of cell loss by NAC was significantly lower in ZnO NPs treated cell groups. Such differences became larger when cells were irradiated at different wavelengths and light intensities, indicating a different cytotoxic mechanism between Zn2+ and ZnO NPs. More detail experimental illustration and results will be presented at the conference. This research was supported by the Environmental Research Center and department of chemistry in Missouri University of Science and Technology.
Understanding molecular transport, i.e., adsorption/desorption, diffusion and migration near a liquid-solid interface is important not only in fundamental sciences but also in advanced applications in separation, heterogeneous catalysis, controlled drug release, trans-cell membrane biological processes, enzymatic reactions, etc. Currently, there is yet no consensus on how far the surface can affect particle diffusing. A number of studies consistently show that the interface only affects water molecules that are in direct contact with, or within a couple of water molecule layers from, the substrate, which indicates the molecule-wall interaction is short-ranged. However, numbers of other observations indicate interfacial effect may be long-ranged, e.g., electrostatic interactions that extend several hundred of nanometers into the solution. High resolution confocal Fluorescence Correlation Microscopy (FCS) has been introduced to study particle diffusion. However, the diffraction limited beam size restricts its application in dynamic studies in nanoconfined environments. Here, we use STED microscopy with a spatial resolution of 45 nm to study highly charged particles diffusing near water-glass interface. In the experiments, unexpected slowdown of particle movement is observed. The unknown dynamics has a time scale comparable to that of free particle diffusion in confocal laser beam, which is not disclosed in conventional confocal FCS. Our study provides valuable insight to fully understand molecular diffusion near interfaces.

Keywords: Absorption, Microscopy, Spectroscopy

Application Code: Nanotechnology

Methodology Code: Fluorescence/Luminescence
In this work, a versatile approach for coupling fluorophores to core-shell silver nanoparticles for metal-enhanced fluorescent cell labels is described. The approach uses physical adsorption of the fluorophore and does not need covalent linkage to create antibody-based labels. We describe the synthesis and characterization of metal-enhanced fluorescence nanocomposites based on core-shell Ag@SiO₂ nanoparticles coupled to rhodamine B. Antibodies were then attached using a non-covalent process that takes advantage of biotin-avidin affinity. Fluorescence intensity was higher in the core-shell nanocomposites than coreless SiO₂ nanobubbles coupled to the same fluorophore and antibody. The enhancement of rhodamine B was between 2.7-6.8 times. We demonstrated labeling of CD19+ Ramos B lymphocytes and CD4+ HuT 78 T lymphocytes using anti-CD19 and anti-CD4 nanocomposite labels, respectively. This physical adsorption process can accommodate a variety of fluorophore types, and has broad potential in bioanalytical and biosensing applications.
Iontophoresis is a drug delivery mechanism by which a small current is used to facilitate the ejection of solute from the tip of a pulled glass capillary. This technique has proved useful in neurological studies since it has the advantage of providing rapid drug delivery to a localized area of the brain. A major limitation to the general adoption of iontophoresis has been the uncertainty in the amount of solute ejected, which ultimately determines the effective concentration of the drug in the region around the capillary tip. As a result, most studies which incorporate this technique are limited to qualitative interpretation. Our lab has previously attempted to quantitate the ejection amount by incorporating an electroactive component into the internal capillary solution and monitoring it at a carbon-fiber microelectrode sealed in an attached capillary. While providing a more accurate estimation of drug concentration, this electrochemical method lacks information on the spatial distribution of the solute.

In this approach we incorporate fluorescent markers, coumarin 120 and disodium fluorescein, into the iontophoretic capillary in order to visualize and quantitate the concentration distribution during and after an ejection. By utilizing dyes of dissimilar charge, we are able to begin to understand the factors that affect the distribution of solute following an ejection. Lastly, fluorescent monitoring is investigated for its possible use with in vitro studies, where the concentration of drug delivered to a brain slice is estimated from the fluorescent intensity of an independent marker.
This research investigates interactions between hydrophobically modified nanoporous silica particles and select surfactants in aqueous solutions. Hydrophobically modified nanoporous silica particles have proven very effective for the containment of drug molecules. However, their hydrophobic coating also introduces challenges in aqueous environments. Modified particles are insoluble in aqueous matrices making them prone to aggregation. Surfactants can be used to solubilize these particles, but a greater understanding of both solubilization and surfactant induced wetting is needed. The structures of various particle-surfactant systems were analyzed using confocal fluorescence imaging. Greater understanding of these interactions will facilitate their future use as drug delivery systems.

**Keywords:** Fluorescence, Imaging, Modified Silica, Nanotechnology

**Application Code:** Nanotechnology

**Methodology Code:** Fluorescence/Luminescence
In recent years, single molecule fluorescence methods have been employed to investigate molecular mass transport in self-assembled one-dimensional nanostructures. Data obtained from such studies have been used to access the diffusion coefficients of single molecules, local nanostructure alignment and order. However, such data are obtained from microscopic sample regions and information from wider sample areas must be obtained from multiple measurements. It is widely believed that the same information can be obtained by ensemble methods but has not been quantitatively compared in one-dimensional nanostructures yet. In this study, the molecular diffusion of sulforhodamine B (SRB) dyes in one-dimensional microdomains of cylinder-forming polystyrene-poly(ethylene oxide) (PS-b-PEO) diblock copolymer films is investigated by ensemble and single molecule fluorescence methods. The diblock copolymer film is prepared between two glass pieces by flowing concentrated polymer solution in organic solvent and drying at low humidity. The diffusion coefficients of single molecules within the flow-aligned PEO domains are determined from single molecule tracking data of individual SRB dyes. The single molecule diffusion coefficients are directly compared to ensemble diffusion coefficients determined from the same PS-b-PEO films.

Keywords: Fluorescence, Microscopy, Nanotechnology, Polymers & Plastics
Application Code: Nanotechnology
Methodology Code: Fluorescence/Luminescence
Enzyme-free signal amplification with DNA circuits has shown great potential in highly sensitive point-of-care molecular diagnostics. Previous works have developed a series of DNA circuits, including hybridization chain reaction, entropy-driven strand exchange and catalyzed hairpin assembly, for detection of proteins and nucleic acids in vitro. However, there have been very few intracellular applications of DNA circuits. Herein, we propose a nonenzymatic DNA circuit for intracellular imaging of low copy-number mRNA with high signal gain. The DNA circuit system is composed of two DNA hairpins (H1 and H2) and a reporter, which is partially hybridized double-strand DNA sequence with a fluorophore and a quencher. In the absence of target mRNA, the two hairpin structures do not hybridize with each other due to the effective block created by complementary domains. The target mRNA first hybridizes with toehold domain of H1 and gradually open the stem of H1 to form mRNA- H1 intermediate, which further hybridizes with H2 to form H1-H2 complex and displace target mRNA. Finally, the hybridized H1-H2 complex displaces the initially quenched fluorophore-modified reporter. In principle, in this system target mRNA sequences play the role of “catalyst” because they facilitate hybridization of H1 and H2 to form H1-H2 complex without being consumed. Compared to molecular beacons and other linear nucleic acid probes, this DNA circuit is capable of generating amplified signal output for detection of low copy-number mRNA targets. This work is supported by grants awarded by the National Institutes of Health (GM066137, GM079359 and CA133086).

Keywords: Bioanalytical, Fluorescence, Imaging, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Peptide Nucleic Acid (PNA) is a synthetic analog of DNA that forms duplexes with itself and with DNA by Watson-Crick base pairing. In contrast to negatively-charged DNA, the backbone of PNA based on aminoethylglycine is neutral. Thus PNA duplexes are more stable than their DNA analogs and are desirable candidates for biosensor technologies and molecular electronics. In this study, a novel scaffold for studying photoinduced electron transfer has been demonstrated by constructing doubly modified PNA duplexes. First, a ruthenium tris(bipyridine) [Ru(Bpy)3]2+ complex was tethered to the PNA backbone as an electron donor and luminescent probe. Second, one of the Watson-Crick base pairs in the duplex was replaced by an 8-hydroxyquinoline pair. We observed that the luminescence from [Ru(Bpy)3]2+* of the modified PNA in solution is largely quenched in the presence of Cu(II). It has been confirmed that this quenching is caused by the formation of copper bis(8-hydroxyquinoline)2 (CuQ2) acceptor which enhances the [Ru(Bpy)3]2+* nonradiative rate by photoinduced electron transfer from [Ru(Bpy)3]2+* to CuQ2. Time-resolved fluorescence studies of the donor-bridge-acceptor molecules as a function of the length and sequence of the PNA bridge and of the position of the donor and acceptor in the PNA showed a distribution of luminescence lifetimes for the donor [Ru(Bpy)3]2+, which correspond to a distribution of electron transfer rates between the donor and acceptor. Molecular dynamics simulations indicate that the distribution of electron transfer rates arise from variations in the conformation of the donor-bridge-acceptor in solution.

Keywords: Fluorescence, Metals, Nanotechnology, UV-VIS Absorbance/Luminescence

Application Code: Nanotechnology

Methodology Code: Fluorescence/Luminescence
In order to gain a better understanding of the microwave synthesis and fully optimize it, we have coupled a CEM Discover S microwave reactor to a Jovin Yvon Fluorolog-3 fluorescence spectrometer via fiber optic cables (set up shown in Figure 1). This allows for the particles to be monitored as they are synthesized. To our knowledge this is the first time that nanoparticle synthesis has been monitored in real-time in a microwave reactor. A representative 3D plot is shown in Figure 2. The x and y axes are emission wavelength and fluorescence intensity, and the z axis is time. Each spectrum represents approximately 1 minute. The initial spectrum is prior to microwave heating.

Using this technique we were able to monitor the fluorescence in real time and quickly determine the optimal temperature and time. We have re-optimized our reaction parameters and improved the quantum yield of the particles from 15% to 19%. This technique shows promise for the fast optimization of microwave reactions involving fluorescent products or reactants, and could be generalized to other forms of spectroscopy for non-fluorescent reactions.


Keywords: Fluorescence, Microwave, UV-VIS Absorbance/Luminescence
Application Code: Nanotechnology
Methodology Code: Fluorescence/Luminescence
Analysis of blood alcohol concentration is a routine analysis performed in many forensic laboratories. This analysis commonly utilizes headspace-sampling, followed by gas chromatography combined with flame ionization detection (GC-FID). Studies have shown several “ideal” methods for instrumental operating conditions, which are intended to yield accurate and precise data. Given that different instruments, sampling methods, application specific columns and parameters are often utilized, it is less common to find information on the robustness of these reported conditions. A major problem can arise when these “ideal” conditions may not also be robust, thus producing data with higher than desired uncertainty or inaccurate results.

The goal of this research is to incorporate the principles of quality by design (QBD) in the development of BAC instrument parameters, thereby ensuring that minor instrumental variations, which occur as a matter of normal work, do not appreciably affect the final results of this analysis. This presentation will discuss both the QBD principles as well as the results of the experiments, which allow for determination of “ideal” instrumental conditions. Additionally, method detection limits will also be reported in order to determine a reporting threshold and the degree of uncertainty at the common threshold value of 0.08g/dL. Finally, differences between pressurized loop headspace systems and volumetric headspace systems will be discussed, comparing and contrasting these two different types of analytical instruments.
Fatty alcohols are used as drug product excipients, food additives, and ingredients in cosmetic products. As these alcohols may be consumed or applied directly to the body, the safety and quality of this class of compounds is important, which is reflected by inclusion of several fatty alcohol monographs in the National Formulary (NF) and Food Chemicals Codex (FCC). As part of ongoing USP-NF and FCC modernization efforts, a GC assay method was developed which allows for separation of 13 alcohols of interest plus an internal standard, ranging from 1-hexanol to 1-docosanol, in less than 15 minutes. The alcohols tested include octyldecanol (branched) and several unsaturated alcohols.

The GC-FID method was developed on a Restek Rtx-225 column (USP phase G7), 30-m x 0.25-mm x 0.25-µm, with hydrogen carrier gas at a flow rate of 2 mL/min. Fatty alcohol samples were prepared at 1.0 mg/mL in ethanol, and the injection volume was 1 µL with a split flow ratio of 100:1. A temperature program from 60° to 220° was used to achieve resolution between all alcohols of interest. The developed method was validated for several fatty alcohols present in the NF or FCC, and the method was found to be specific, accurate, precise, and linear. The validated method will be presented for public comment in the respective monographs for cetyl alcohol, stearyl alcohol, and cetostearyl alcohol in the Pharmacopeial Forum (PF) in November 2013, and additional monograph modernizations for these fatty alcohols will be presented in future editions of the PF.

Keywords: Capillary GC, Gas Chromatography, Pharmaceutical, Validation
Application Code: Pharmaceutical
Methodology Code: Gas Chromatography
California recently enacted legislation to mandate monitoring of trace impurities in hydrogen fuel that is commercially sold in California for vehicle use. Some of the target analytes can have severe impact on performance of fuel cells used to power vehicles. Others are included to ensure the fuel has not been diluted with air or other gases. California Department of Food and Agriculture, Division of Measurement Standards is assigned the task to develop test methods for measuring these impurities. California State University, Los Angeles, is constructing a fueling station for public access, and is using analytical methods similar to those at the Division of Measurement Standards for testing the fuel purity.

The paper reports on the design of instrumentation to measure most of the gaseous constituents and performance achieved in meeting the mandated fuel specifications. The list requires use of multiple detectors to attain detection below the required action levels, thus allowing a comfortable margin to proper assess the fuel. Included are:

- Flame ionization detector for Total Hydrocarbons
- Ring-down spectrometer for Water and Formaldehyde
- Electron capture detector for Oxygen
- Thermal conductivity detector for Helium
- Pulsed discharge detector for Nitrogen and Argon
- Flame ionization detector with reduction catalyst for Carbon Monoxide and Carbon Dioxide
- Pulsed flame photometric detector for Total Sulfur Compounds
- Mass spectrometer for Total Halogenated Compounds

Results are given for measured linearity and detection limits for the low-level analytes. Special operations to properly handle the high pressure sample canisters are presented.

Keywords: Analysis, Fuels\Energy\Petrochemical, Gas, Gas Chromatography
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography
## Abstract Text

Ionic liquid–static headspace single drop micro extraction (IL-SHS SDME) involving three phase equilibrium was performed to extract aromatic hydrocarbons from water. In IL-SDME, a single drop of ionic liquid is suspended from a syringe needle and exposed to the headspace in a sealed vial containing a sample. To improve extraction efficiency an ultrasonic bath was used. A quick extraction was performed by taking the analyte in a 2 ml vial with 0.5 ml headspace volume. The extraction time was 8.0 min. Two ionic liquids, 1-Butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide and 1-Butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, both having same anion were examined. A direct, no interface introduction of ionic liquid into the gas chromatographic inlet was performed. Ionic liquid stationary phase was used for the chromatographic separation and GC MS for instrumental analysis. The partition coefficient of the aromatic hydrocarbons between the ionic liquid and water was determined by depletion study. A plot of log peak area was made against extraction number to determine the extraction ratio and partition coefficient. The partition coefficient was higher for the aromatic hydrocarbons when 1-Butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide was used as the extracting micro droplet. The extraction method was then extended for quantitative analysis. The method presented %RSD for precision lower than 5%, recovery of 88.9% to 98.1% and the limit of quantitation was 0.06 ng L\(^{-1}\). Real samples of drinking water were collected from different source and aromatic hydrocarbons were not detected in any of them. Ionic liquid based microextractions not only served as ‘green’ alternative to traditional extraction but also proved to be selective, efficient and time saving sample preparation and pre-concentration technique.

### Keywords
- GC-MS
- Quantitative
- Sample Preparation
- Validation

### Application Code
- Environmental

### Methodology Code
- Gas Chromatography
The prediction of retention in gas chromatography has applications ranging from aiding in the identification of molecules to the optimization of experimental conditions. While the retention index is historically the most popular approach to retention prediction, there has been a recent resurgence in the use of thermodynamic modeling. Recently, an approach to rapidly obtain thermodynamic parameters of $H(T_0)$, $S(T_0)$, and $C_P$ for the interaction of a molecule with the stationary phase in GC has been demonstrated. Additionally, a method to account for the variability in column geometry inherent to the manufacturing process has been developed. Results of an inter-laboratory trial to evaluate these approaches to modeling GC retention time will be presented. The trial involves five laboratories on three continents testing a suite of molecules spanning a range of different chemistries and degrees of complexity on three stationary phase chemistries.

**Keywords:** Gas Chromatography, GC, Separation Sciences

**Application Code:** General Interest

**Methodology Code:** Gas Chromatography
Determination of sulfur containing components in different petroleum feeds and products is a true analytical challenge; since they can occur in very complex matrices and often in widely varying concentrations. Moreover, they are highly reactive and have adsorptive and metal catalytic properties. Therefore, analysis of sulfur compounds requires the sample pathways including column to be inert to ensure reliable results. Gas chromatography with Sulfur Chemiluminescence detection (SCD) has become the tool of choice to identify and quantify various sulfur compounds in different petroleum samples. But if columns with higher bleed profile installed on the SCD, can contribute to detector overloading in which hydrocarbons/aromatics as well as components of stationary phase bleed exiting the column are reduced in the hydrogen rich atmosphere which “coke” SCD reaction tubes in the burner. This inadvertent passivation of the reaction tubes in turn causes a rapid decline in the SCD sensitivity. The only solution is to replace the reaction tube, which results in instrument down time and increased cost of operation. A novel wall coated open tubular column with low bleed and exceptional inertness to sulfur compounds enables separation of a broad range of the active solutes at even low concentrations. Evolving column technology allows for not only analyzing light sulfur gases but also extending to sulfur containing hydrocarbons out to C20:. Good resolution and peak shape can be achieved. Performance for SCD demonstrates minimal column contribution to reaction tube coking which results in improved system ruggedness and greatly reduced downtime for detector maintenance.
Interest in microfabricated GC (GC) has endured through the past 34 years because of its potential for providing rapid analysis at any point of sampling. Sample loss, contamination and decomposition that often accompanies sample transport and storage can be avoided by deploying GC in the field. Despite these advantages, GC suffers from relatively poor separation performance, i.e., column efficiency can be compromised by non-uniform stationary phase coatings, peak shape distortion can occur when dead volume or active sites exist, manual injections often compromise the analytical results when an auto-injector is not available, and large sample volumes required for trace analysis can reduce resolution. Fortunately, these effects can be minimized or even eliminated by applying a negative temperature gradient along the column. A broad sample band is narrowed in a thermal gradient by slowing down the front portion of the peak, which is at a lower temperature, and accelerating the back portion, which is at a higher temperature. To demonstrate the potential of thermal gradient GC (TGGC), we fabricated all-silicon micro columns using photolithography, wet etching and dynamic coating techniques. Thermal gradients were generated using planar heaters attached to the wafers. Compared to isothermal and programmed temperature GC, TGGC preserves column efficiency and resolution by counteracting already existing capillary GC challenges that are magnified with microfabricated columns. With precise control of the gradient, GC should approach its full potential and become a great tool for volatile chemical analysis.

Keywords: Gas Chromatography, Lab-on-a-Chip/Microfluidics, Portable Instruments, Temperature
Application Code: Environmental
Methodology Code: Gas Chromatography
Abstract Text

The typical chromatographic methods for analyzing fatty acids and neutral lipids are gas chromatography after derivitization and liquid chromatography-tandem mass spectrometry (LC/MS/MS). However, there are shortcomings associated with each of these methods. For example, GC methods require derivatization of the fatty acids to methyl esters (FAME), which is burdensome, time consuming, and there is a risk of re-arrangement of the fatty acids during derivitization which leaves doubt as to whether the esters formed are from free fatty acids or intact complex lipids. In LC/MS/MS methods, the runs typically involve labor intensive and time consuming sample preparation, and utilize toxic solvents, which are expensive to purchase and dispose. We have developed rapid (5min), high throughput and efficient method for the separation and analysis of free fatty acids and neutral lipids without derivitization using sub-2µm particle CO2 based supercritical chromatography. The organic extract from the matrix containing lipids is directly injected onto the system showing a significant saving in solvent, cost and sample preparation time. The separation mechanism is mainly based on the number of carbon chains and the number of double bonds on the acyl chain. The datasets were processed using TransOmics Informatics for Metabolomics and Lipidomics a new software tool that provides automatic peak detection followed a quantitative comparison and statistical analysis to differentiate those features that are significantly changing and finally identify those features from the mass spectrometry data.

Keywords: Lipids, Metabolomics, Metabonomics, SFC, Time of Flight MS
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Separation Sciences
Xenopus laevis is an important model organism for studying early vertebrate development. While much information is available on the temporal changes of mRNA expression in early Xenopus embryos, there have been very few corresponding studies addressing changes in protein expression. We report the first large-scale qualitative and quantitative proteomic analysis of single Xenopus embryos. We employed iTRAQ 8-plex labeling of peptides, two-dimensional liquid chromatography (offline SCX-RPLC) separation, and high-resolution tandem mass spectrometry (Q-Exactive) identification. Over 4,000 proteins were identified with a protein-level false discovery rate (FDR) of 0.3% or less. The abundance change of 3,983 proteins across at least four developmental stages was obtained. The protein expression change mirrors the major regulatory changes in transcription that occur during early Xenopus development. Proteins encoded by maternal mRNA present in the egg can be discerned as well as those solely encoded by zygotic mRNA. Organ and system specific proteins appear following the transcriptional reprogramming that occurs at stage 13. However, our analysis also reveals that there is an appreciable amount of post-transcriptional regulation that controls expression of maternal mRNAs beyond the mid-blastula transition that allows for a seamless progression into the more complex patterns of zygotic transcription. We also determined there is no evidence for significant protein heterogeneity between individual embryos at the same stage of development. These Xenopus embryos proteome data sets are the largest reported to date. This project was supported by a grant from the National Institutes of Health (Grant R01GM096767).
The chemical fingerprint of an individual's urine can provide critical information about their diet, use of medications or illicit drugs, and overall health. However, a majority of published work to date focuses on a targeted approach where only select compounds are investigated. In this work, we use LC × LC to develop a method for the untargeted analysis of urine to produce chemical fingerprints. Based on our previous experience with LC × LC, we are utilizing a carbon clad silica column in the second dimension. For the first dimension column, we examined the potential of a C18 column and a mixed mode hydrophobically assisted weak cation exchange (WCX) column to provide sufficient separation. We also compared the effect of using mobile phase pH as a variable on each column. Our preliminary results indicate that the C18 column is capable of providing better resolution than the hydrophobically assisted WCX column. We also investigated the use of sodium perchlorate and sodium hexafluorophosphate as ion pairing reagents. However, we only saw a slight improvement in peak resolution on the C18 column. We are currently investigating the use of a hydrophobically assisted strong cation exchange column in the first dimension.
Detection of small organic compounds (< 1500 Da) in water column could be very useful in the research area of marine chemistry to improve our knowledge on ecosystem dynamics (e.g. chemical communication between organisms). Removing water samples or living organisms from their environments may be critical since changes in physical and chemical properties (e.g. pressure, temperature and salinity) of samples can affect the biological equilibrium, leading to loss and/or degradation of analytes. The current study introduces in vivo Solid-Phase Microextraction (SPME) as a new technique for underwater extraction of a wide range of analytes. In vivo SPME sampling were performed for extraction of small molecules profiles present in water surrounding corals. Analyses were conducted with a LC-MS method using a benchtop orbitrap instrument in both positive and negative modes.

A total of 283 unique compounds were detected. Following comparison of peaks against compounds candidates available from database (HMDB and Metlin), 91 unique compounds were tentatively identified. Most of these compounds belong to eicosanoids, steroids, prenol lipids, glycerophospholipids, fatty acids and glycerolipids. The partition coefficient (log P) of compounds tentatively identified ranged from -2.7 to 15.3. Of the list of compounds identified, five were short-lived intermediates.

The result of our study showed that it is possible to extract from underwater more than hundreds of compounds with a wide range of polarities simultaneously, including short-lived compounds, by using small SPME fibers.
Many analytical techniques, especially spectroscopic and chromatographic, provide numerous data points for each sample analyzed. For example, a typical GC-MS analysis can easily generate 8 million data points per hour. Herein, a custom-written algorithm was compared with some of the more common commercial variable selection techniques. The custom-written algorithm used cluster resolution to guide a combined backward elimination/forward selection approach with either ANOVA or selectivity ratio (SR) as the variable ranking metric. The comparison was based on the development of PLS-DA models. Three different metabolomic data sets from three different analytical platforms (ATR-FTIR spectra, GC×GC-TOFMS, and LC-QTOFMS) were used for the study. The study compared a variety of feature selection and ranking techniques based on factors such as quality of the “optimised” model identified in each case, ease of automation, computation time, efficiency of data reduction, etc. The lab-built algorithm showed better performance in terms of maximal predictive capability, with minimal computational time, and minimal analyst effort.

Keywords: Chemometrics, Chromatography, FTIR, Mass Spectrometry
Application Code: Genomics, Proteomics and Other ’Omics
Methodology Code: Chemometrics
The MRC-NIHR National Phenome Centre, Imperial College London, is the first of its kind facility. Born out of the UK Olympic Legacy its mandate is to provide “high throughput, forensic quality, metabolic phenotyping to support large scale epidemiological studies as well as basic medical research into disease understanding and patient stratification”. As global life-styles change we are seeing increasing cases of obesity, diabetes, and mental health issues. This not only affects a person’s quality of life but also places increased strain on the health-care systems to provide the right treatment whilst managing costs closely.

Metabolic Phenotyping offers a valuable and unique insight into the underlying biochemistry of diseases as well as the patient’s individual biochemistry “phenotype”, diet, health status, age and stress. To deliver this information the analytical data generated is processed via a variety of chemometric modelling and analysis methodologies to deliver the relevant biochemical information. These chemometric platforms employed vary from simple multivariate analysis to highly complex model based analysis and is presented in a format ready for interpretation by medics.

This facility comprises of high field NMR instruments, accurate mass LC/MS instruments, tandem quadrupole LC/MS systems as well as dedicated training facility. In this presentation we will discuss the development of analytical platforms both LC/MS and NMR as well as a detailed discussion on the workflow, validation, reporting and decision making process. The presentation will cover the development and validation of the “discovery’ screening methods for polar, non polar metabolites and lipid profiling using LC/MS methodology, as well as describe the use of proton NMR as an initial screen to eliminate contaminated samples. The quantitative targeted LC/MS assays will also be discussed the various compounds classes such as bile acids, amino acids, eicosanoids, and acyl carnitines.

Keywords: Biomedical, Lipids, Liquid Chromatography/Mass Spectroscopy, Metabolomics
Application Code: Genomics, Proteomics and Other ’Omics
Methodology Code: Liquid Chromatography/Mass Spectrometry
The recent CFE 100+ initiative at the University of North Carolina at Chapel Hill was designed to improve student engagement in lecture courses with over 100 students. Traditional sections of the sophomore-level analytical course at the University usually enroll over 200 students, so the use a suggested CFE 100+ method was explored. A common technique for increasing interaction in large lectures is with the use of student response systems, or “clickers”. However, standard clicker systems traditionally require initial purchases by students and additional hardware and software for the instructor. Alternatively, online response systems like Poll Everywhere can be utilized by any student with Internet access (through a mobile device or laptop) or text messaging capabilities. Responses are then available through an easy-to-use web interface, so no extra investment is required by the instructor. In this activity, students were invited to non-mandatory review sessions prior to each course exam. Throughout the review, students were able to respond to practice questions through Poll Everywhere so that real-time feedback could be given on each test item. This enabled targeted discussion on topics that were confusing to a majority of the students. This presentation will describe how the initial stages of the activity were accomplished and how students responded to the review sessions.
For over 15 years, roughly every student taking the second semester senior Chemical Instrumentation course at Hampden Sydney College has attended Pittcon as a part of the course. Far from being limited to an "enrichment experience", the Pittcon portion of this course has a substantial academic component to it. Each student is assigned an instrument that is not currently in our departmental stable. Before Pittcon, students must learn its physical operating principle, the information it produces, the systems it has found use in and where our department might incorporate that instrument in our curriculum. During the Exposition, they must contact exhibitors of that instrument, get answers to questions regarding operation, routine operating costs, times, consumables, site requirements, etc. At the end of the term, they write a proposal to the department and present a seminar making their recommendation. Not only has the department benefited from this guidance in selection of instrumentation, most of these students eventually have had to recommend, approve or specify instrument purchases in their professional life.
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 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.

Keywords: HPLC, Method Development, Pharmaceutical, Teaching/Education
Application Code: Other (Specify)
Methodology Code: Education/Teaching
Abstract Text

Chromatography is an important enabling analytical methodology that is used in various forms across the chemical and biochemical disciplines for a wide variety of applications, including pharmaceutical, food, environmental and biomedical analyses and studies. It is therefore important that it also be a significant component of undergraduate education in science, especially in chemistry and biochemistry. Because of time and cost limitations generally associated with high performance liquid chromatography (HPLC), most universities with large enrollment laboratory courses have avoided this technique in favor of gas chromatography. Liquid chromatography, however, has significant pedagogical advantages over gas chromatography because students can be presented with physical and visual examples of liquid chromatographic separations and the absorbance spectroscopy utilized for HPLC detection in simple bench-top experiments.

In this project, recent advancements in HPLC technology have been utilized to introduce hands-on exercises demonstrating separations of simple mixtures in the undergraduate laboratory. An important advancement of this project is the implementation of liquid chromatography exercises in large enrollment laboratory courses like those taught at most intermediate or large universities. This is accomplished through early instruction using illustrative bench top exercises, through application of state of the art yet affordable separations instruments and technology that allow fast separations, and through the use of multiple instruments available to each laboratory section.

Keywords: Education, HPLC, Instrumentation, Liquid Chromatography
Application Code: General Interest
Methodology Code: Education/Teaching
The cost of corrosion-related failures in nuclear power plants (NPPs) is significant. In pressurized water reactors, deposit fouling in the secondary system can cause undesired consequences. Studies show that the addition of polyacrylic acid (PAA) to the steam generator water increases the corrosion resistance of steel and promotes removal of iron-based deposits from surfaces. Since 2009, PAA has been used at several NPPs as a dispersant in the feed water. These NPPs need to accurately measure PAA to ensure that the added amount is within the plant’s administrative limits. The NPPs also need to determine trace concentrations of ionic impurities, which are critical for the identification and prevention of corrosive conditions in power plant components. The feed water in the secondary system contains other corrosion inhibitors (e.g., ethanolamine [ETA]) to obtain an alkaline pH and a volatile oxygen scavenger (e.g., hydrazine) to form protective magnetite on the surfaces of the steam generator components. We present a size exclusion chromatography method to determine low (< 20 µg/L) concentrations of PAA and an ion chromatography method to determine trace concentrations of fluoride, chloride, and sulfate in secondary feed water samples containing PAA, ETA and hydrazine. PAA is resolved from the matrix on a polymer-based size-exclusion column using water as the eluent, and detected at 200 nm with a detection limit of ~2.6 µg/L. The trace anions are extracted onto a concentrator column while the sample matrix is diverted to waste. The anions are then separated on an anion-exchange column using isocratic elution with electrolytically generated potassium hydroxide and detected by suppressed conductivity. The recovery of spiked PAA and anions of interest in the secondary feed water and simulated secondary feed water are in the range of 99.5-109% and 92.3-101%, respectively.

**Keywords:** Ion Chromatography, Liquid Chromatography, Nuclear Energy, UV-VIS Absorbance/Luminescence

**Application Code:** Nuclear

**Methodology Code:** Liquid Chromatography
This talk describes the evolution of instrumental and chemometric methods implemented to monitor plutonium reprocessing operations at US DOE’s Savannah River Site. Relatively simple initial monitoring requirements supported the use of filter photometers to detect Pu(IV) elution from a separation column, triggering automated valve switching. The elution process includes wide swings in nitric acid concentration, altering the distribution of Pu(IV) nitrate complexes. Each complex has a unique and complicated absorption spectrum, and these spectra overlap considerably. Through a first-principles instrument simulation, we were able to determine a filter set which allowed univariate Pu measurement within 8-10%. Subsequent expansion of the monitoring scope led to conditions with new acidities, where not only is the nitrate complexation different, but significant disproportionation of Pu(IV) to Pu(III) + Pu(VI) occurs. We have demonstrated that filter selection cannot be altered to measurement accuracy in this scenario. Accordingly, we have developed methods based on chemometric analysis of full absorption spectra that can report total Pu concentrations and distributions of individual oxidation states, as well as nitric acid concentration and potential transition metal interferents. We use a graded analysis, where initial classification permits secondary selection of precise localized models. We also describe a new implementation of the diode array spectrophotometer that yields increased stability and sensitivity at competitive costs. These sensors, which can be easily adapted to other actinides, could be a useful tool to detect material diversion and promote nuclear safeguards.
Radioactive strontium-90 scattered by nuclear power plant accident was specifically quantified by conventional inductively coupled plasma quadrupole mass-spectrometry (ICP-QMS) preceded by on-line chelate column separation (based on lab-on-valve) and oxygen reaction (designated the cascade step). The proposed system overcomes the isobaric interference of $^{90}\text{Zr}$, whose soil concentration exceeds that of $^{90}\text{Sr}$ by more than six orders of magnitude. In addition, the system requires no ultimate mass spectrometry or radioactive $^{90}\text{Sr}$ standards. The radioactive $^{90}\text{Sr}$ standard was replaced with the stable isotope $^{88}\text{Sr}$ as a pseudo-standard. The modified ICP-QMS system yielded a precise, reproducible sharp $^{90}\text{Sr}$ peak in the ICP-MS profile. The elution time of $^{90}\text{Sr}$ was highly reproducible (RSD = 0.5 %). After implementing the cascade-step, the detection limit (DL) was 2.3 Bq/L. Analysis of microwave-digested soil yielded a DL of 3.9 Bq/kg. The $^{90}\text{Sr}$ from environmental contaminated soil samples collected from areas at a distance of 10 and 20 km from the Fukushima daiichi nuclear power plant ranged from 52 Bq/kg to 73 Bq/kg, with no statistical difference between the proposed and general methods at 95% confidence level. The proposed method offers an attractive alternative use for ICP-other ionization mass spectrometry as an enrichment or purification step, thereby expanding the scope of ICP-mass-spectrometric analysis.

Keywords: Environmental Analysis, ICP-MS, Nuclear Analytical Applications, On-line
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Nuclear power plants produce a significant portion of the electrical energy globally. These power generators must be properly maintained to supply continuous, uninterrupted electrical energy to meet consumer demands. The buildup of impurities in water used to drive steam generators and turbines can cause corrosions that lead to component failures and plant shutdowns, resulting in significant loss of power generation. Hence, monitoring the presence of ionic impurities in cooling waters, boiler waters, feed waters, and steam condensate in nuclear power plants is critical to the operation of power generators in the nuclear power plants. Ion chromatography (IC) is an indispensable technique for the determination of trace concentrations of ionic impurities found in nuclear power plant waters.

The use of capillary IC systems has gained increasing interests in recent years. Compared to a conventional IC system using 4-mm ID separation columns, a capillary IC system using 0.4-mm ID separation columns provides increased mass sensitivity for target analytes while maintaining the concentration sensitivity for target analytes. This important feature of capillary IC systems can be used to improve the determination of target analytes at trace levels since the sample volume can be reduced by a factor of 100 to achieve same concentration sensitivity. Instead of loading 20 mL of a sample onto a concentrator column via a loading pump as it is commonly practiced in a conventional IC system, a 200-μL of sample can be injected onto a concentrator column much more easily and conveniently in a capillary IC system. In this presentation, we will discuss several approaches of using capillary IC systems with on-line electrolytic eluent generation and suppressed conductivity detection for determination of anionic analytes at trace concentrations in nuclear power plant waters.

Keywords: Ion Chromatography, Nuclear Energy, Sample Preparation, Water
Application Code: Nuclear
Methodology Code: Liquid Chromatography
Atomic Force Microscopy (AFM) has been a widely utilized tool in both industry and academia for imaging the surface of a material with nanoscale resolution. Today’s polymer blends and composites are more complex than ever, leading to challenges in characterizing the distribution of different components in the blend or composite. Lorentz Contact Resonance (LCR) allows for the clean excitation of the contact resonance modes of a Thermalever™ AFM cantilever. The contact resonant frequency and amplitude of these resonances are dependent on the stiffness of the material in contact with the probe. When tuned to a particular resonant frequency and scanned across a sample, the Thermalever probe can obtain a qualitative map of the varying stiffness of each component on the surface of a sample. Each individual component can then be highlighted by tuning to its resonant frequency and scanning the surface. This results in the visualization of the location and size of the domains of each component in a polymer blend or composite, greatly increasing our understanding of how these blends form. Furthermore, the Thermalever probes are fabricated with a resistive heating element at the end of the probe allowing for the controlled heating of the probe. Once a domain in a blend or composite has been identified using the LCR technique the Thermalever probe can then be placed on the region of interest and nanoscale thermal analysis (nanoTA) measurements such as glass or melt transition temperatures can be obtained.

Keywords: Materials Characterization, Polymers & Plastics
Application Code: Polymers and Plastics
Methodology Code: Surface Analysis/Imaging
Determining the molecular surface and bulk effects short wave UV light has on bis-2-ethylhexyl phthalate (DEHP) plasticized PVC is vital for both green chemistry (removing toxic plasticizer from plastic after disposal in an energy-efficient manner) and environmental purposes (to better understand what molecular changes occur to DEHP and PVC from UV light exposure by lamps or sunlight). Using a combination of analytical techniques including sum frequency generation vibrational spectroscopy (SFG), FTIR, and secondary ion mass spectrometry (SIMS), we analyzed the surface and bulk of 10 or 25 wt% DEHP-plasticized PVC before and after a variety of short wave UV treatments from 30min to 8h in length. It was determined that 254 nm UV exposure at 53 W/m[sup]2[/sup] yielded bulk products phthalic acid, mono-ethylhexyl phthalate and phenyl ring hydroxylated DEHP as well as smaller molecules after only 1h exposure time. These molecules were also present on film surfaces. In contrast, the up-and-coming green method for DEHP removal, short wave UV light and oxygen radicals from 35 wt% H[sub]2[/sub]O[sub]2[/sub] solution, eliminated fewer bulk DEHP molecules up to 8h due to competing radical-polymer reactions, but began degrading surface DEHP molecules in 30min rather than 1h. We are currently obtaining SIMS results for molecular surface products after 5 and 8h of the H[sub]2[/sub]O[sub]2[/sub]/UV treatment. Our results indicate a simple exposure to short wave UV for several hours is more effective at eliminating bulk DEHP while H[sub]2[/sub]O[sub]2[/sub]/UV combination should be used for surface treatments only. Additionally, we can extrapolate that exposure to short wave UV over a plastic’s lifetime will yield surfaces containing a variety of stable DEHP-related molecules accessible to contact with living organisms before they are completely removed from repeat UV exposure.
Compounding is a critical step in the manufacture of a material – arguably as critical as synthesis. In particular, compounding extrusion is done in the molten state by blending one or more polymers with selected additives. The high temperature required to melt the polymers and the heat generated by viscous dissipation creates an environment in the extruder where the chemical makeup of the ingredients can be altered – potentially the cause for subpar material performance. In the case where several polymers are being blended, the compounding conditions may represent a compromise between that of the different polymers, exposing the weaker component to conditions more extreme than desirable. The compounding conditions may also be designed so as to promote the reaction between two or more ingredients. This is known as reactive blending and it is a very practical tool to achieve compatibilization of two polymers via the in-situ formation of a block copolymer. Once blended it may be difficult to obtain information about the individual components and to diagnose their condition. In this paper, several examples of compounding involving multiple polymers are examined. In particular, the high resolving power of two-dimensional chromatography is exploited where the first dimension provides separation based on composition (GPEC) and the second on molecular weight (GPC). This enables us to shed a new light into the mechanisms at play during compounding in order to identify the weak link in a formulation or the efficiency of an additive.

**Keywords:** Chromatography, HPLC, Materials Science, Polymers & Plastics

**Application Code:** Polymers and Plastics

**Methodology Code:** Separation Sciences
Gold nanohole arrays are a plasmonic sensor having a great potential toward high throughput biosensing. Indeed, nanohole arrays require simple optical setup working in transmission spectroscopy, benefit from the availability of low-cost manufacturing technologies and exhibit high sensitivity. Such sensitivity of gold nanohole arrays towards surface and bulk refractive index varies depending on structural and optical parameters. Gold nanohole arrays supports surface plasmon polaritons (SPPs), which 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 wavelength shift, in comparison to direct transmission analysis, for the detection of IgG (limit of detection of 5 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). Optimal excitation angle of nanohole arrays gave an additional 20% amplification signal of 4-NBT in SERS compared to normal angle. In conclusion, gold nanohole arrays hold great promise for the development of a future multiplexed plasmonic transducer.

Keywords: Biosensors, Biospectroscopy, Nanotechnology, Sensors
Abstract Text

Interactions between solution phase analytes and surface immobilized biorecognition elements in heterogeneous assay formats, such as ELISAs, are often hindered by mass transfer limitations resulting in suboptimal detection limits and prolonged assay times. Magnetic nanoparticles overcome some of the limitations with respect to analyte capture due to their relatively large surface areas and ease of mixing within sample matrices. However, sandwich complex formation remains hindered by mass transfer limitations of the labeled detection species with the captured analyte. As demonstrated here, these limitations can be overcome by rendering the signaling species magnetic. Specifically, a ferromagnetic metal oxide was incorporated into the lipid bilayer of fluorescent dye-encapsulating liposomes, allowing for influence under a magnetic field while maintaining a high capacity for encapsulation of signaling molecules within the interior volume. Liposomes were synthesized encapsulating sulforhodamine B, with bilayers functionalized with both cholesterol-modified DNA reporter probes and oleic acid-Fe3O4 conjugates. These liposomes were magnetically directed towards binding surfaces; magnetically retained in flow-based microfluidic devices; and ultimately utilized as superior signaling reagents. Using these magnetically influenced fluorescent liposomes, the limit of quantification was decreased by a factor of 15 in the presence versus absence of an underlying magnet in a sandwich-hybridization microtiter plate-based assay for Cryptosporidium parvum DNA/RNA. These liposomes also yielded a reduction in assay incubation times and lower reagent concentrations. These benefits resulted despite using only a suboptimal magnetic set-up at present. The superior performance of these magnetic liposomes as bioanalytical signaling reagents and their adaptability to microtiter plate, microfluidic formats, and sample preparation procedures offers many exciting detection opportunities.

Keywords: Bioanalytical, Fluorescence, Immunoassay, Nucleic Acids

Application Code: Bioanalytical

Methodology Code: Sensors
### Abstract Text

Fast and simple DNA detection has always been an important goal for DNA sensor research. Numerous DNA detection methods have been developed and commercialized, based on fluorescence, electrochemistry, PCR, etc. The challenge of the field is now focused on finding the optimum balance between sensitivity, convenience, efficiency and cost. In this presentation we report our recent progress on the development of a field-friendly, sensitive, and inexpensive DNA screening method based on the Amplification-by-Polymerization technique. Specifically, we have successfully carried out Reversible Addition-Fragmentation chain Transfer polymerization (RAFT) reaction at room temperature in open air for DNA detection. Different monomers and initiator systems have been evaluated and polymerization reaction conditions optimized. This polymerization reaction was applied to grow polymer on gold substrate bonded DNA and small molecular and the kinetics of the surface polymerization was studied. Reaction kinetics of polymer was studied for both SAM-coated and DNA coated substrates. DNA detection sensitivity is formed to be comparable to previous reported result where RAFT is conducted under protected environment. Also, the reaction of the aqueous RAFT polymerization in open air have been carefully studied. We have compared the effect of the stirring, initiator concentration, reducing agent amount and monomer type and concentration for the room temperature aqueous RAFT polymerization.

The report work of surface polymerization lays a solid foundation for the development of simple, cheap and fast sensor device. This room temperature, open air aqueous polymerization can be applied for the sensitive biomolecular detection such as DNA, RNA or protein and may have further applications.

### Keywords
- Bioanalytical
- Biosensors
- Polymers & Plastics
- Sensors

### Application Code
- Bioanalytical

### Methodology Code
- Sensors
The successful ultrasensitive biosensing was achieved for the detection of pandemic virus, bovine viral diarrhoea virus (BVDV) type 1 (BVDV) by using dual ssDNA aptamers successfully developed by immobilization-free screening method. Aptamers are single-stranded nucleic acids having molecular recognition properties similar to antibodies, and isolated by in vitro selection and amplification process, SELEX. In this study, a new immobilization-free aptamer screening method is developed by exploiting the properties of graphene oxide (GO) to adsorb ssDNAs (1). The target induced conformation change of aptamers initiates the affinity based desorption of ssDNAs from the GO. For the target, BVD virus, GO-SELEX required less than five SELEX rounds to obtain aptamers with high affinity and specificity. This modified GO-SELEX was resulted in developing the aptamers that bind to the whole virus with high affinities. Two selected sequences successfully obtained were found to have different binding sites and so can be used in a sandwich-type assay format for the sensitive detection of BVDV type 1. The very sensitive limit of detection with 500 TCID50/ml is comparable to the commercialized conventional real time PCR kit.
**Abstract Text**

The sliver sensor is a miniature optical sensor array consisting of multiple sensing wells to measure different metabolic analytes in vivo in the skin. Since the array is implanted in the skin close to the surface, the colors of the sensing wells are visible from outside, however to an extent distorted due to absorption and scattering in the skin in both directions. The true colors as would be visible directly at the sensors’ surface thus need to be reconstructed from colors seen from outside the skin to provide the resolution needed to monitor changes in the interstitial concentration of analytes over time.

The skin was modeled in this work using a lightly colored scatterer layer. Red, blue, green, white and black color-standard plastic pieces were placed under this layer to model the sensor array. The experiments were performed with no hydrogel, hydrogel that only scattered light, a layer of light color without scattering, and a composite layer with scattering and color. A theory based on multiple reflections of direct light and light back-scattered in both directions has been derived using the white and black optical references for color reconstruction. Results show that colors close to the colors of the standard spots can be reconstructed this way. Initial tests in animal skin suggest that the approach can work also in vivo.

**Keywords:** Biosensors, Chemometrics, Data Analysis, Imaging

**Application Code:** Biomedical

**Methodology Code:** Sensors
Matrix proteases break down extracellular matrix material and play a key role in many diseases such as periodontitis, osteoporosis, arthritis, and cancer invasion. Studying these proteases’ activity is essential to understanding diseases and developing therapeutics. We have developed a simple and effective method to detect the proteolysis event using magnetically modulated optical nanoprobes (MagMOONs). For demonstration, we use alginate calcium matrix as the substrate that is lysed by alginate lyase. The MagMOONs are micron sized hemi-spherically metal-coated fluorescent probes having orientation-dependent fluorescence. These particles give blinking signals in accordance with an external rotating magnetic field. The blinking signals from these MagMOONs are distinguished from background scattering or auto-fluorescence from tissue, and can be tracked in situ and at low magnification. In this study, MagMOONs are at first trapped in an alginate calcium matrix and therefore give no response to magnetic modulation. After alginate lyase is added, their modulated signal indicating alginate matrix cleavage. The blinking is clearly detected both in vitro and in real time in situ under up to 4 mm chicken breast. Our results show MagMOONs start giving a blinking signal after approximately 10 minutes following 2 mg/mL alginate lyase addition. A further step will be co-encapsulating these sensors with drugs in gel so that when the gel matrix is cleaved, both of detection and treatment purposes are addressed at once.

Acknowledgment. This work was supported by the South Carolina Research Authority NSF grant 2002-593TO#0056; NIH NIBIB grant, award number 1R15EB014560-01A1, and a Vietnam Education Foundation (VEF) fellowship to KVTN. We thank Spherotech for generously donating the magnetic fluorescent particles.

Keywords: Bioanalytical, Fluorescence, Imaging, Microscopy  
Application Code: Bioanalytical  
Methodology Code: Sensors
We developed novel air-stable 2D polymerized photonic crystal (2DPC) sensing materials for visual detection of gas phase analytes by utilizing an ionic liquid as the dispersing medium. Ionic liquids have very low vapor pressures, preventing their evaporation. We designed Ionic Liquid 2DPC (IL2DPC) humidity and ammonia sensing materials that utilize a new ionic liquid, ethylguanidine perchlorate (EGP) as the dispersing medium for poly(hydroxyethyl methacrylate) (pHEMA)-based polymer networks that have a 2D array of polystyrene nanospheres embedded onto the polymer. The bright 2D photonic crystal diffraction depends sensitively on the 2D array particle spacing. The volume phase transition response of the polymer to water vapor or gaseous ammonia changes the IL2DPC particle spacing, enabling visual determination of the analyte concentration. Water absorbed by EGP increases the polymer-solvent interaction parameter between pHEMA and EGP, shrinking the IL2DPC and causing a blue shift in the diffracted light. IL2DPC was functionalized to detect ammonia by introducing the pH sensitive co-polymer, acrylic acid (AA). Ammonia absorbed by EGP deprotonates the carboxyl group of the pHEMA-co-AA polymer, swelling the IL2DPC and causing a red shift in the diffracted light.
Solid phase micro-extraction (SPME) is an important sample pre-treatment technique which enables to concentrate the target analyte so as to meet the requirement of detection sensitivity. In this presentation, we like to present several new approaches for developing highly sensitive SPME techniques in our group. The first approach is that we developed a technique by universal multilayer assemblies of graphene in chemically resistant micro-tubes, such as polytetrafluoroethylene (PTFE) and poly(ether ether ketone) for microextraction of polyaromatic hydrocarbons (PAHs) [1], the method has been applied to determine PAHs in environmental samples with exceptional sensitivity. The second approach is mussel-inspired polydopamine functionalized poly(ether ether ketone) tube for online solid-phase microextraction-high performance liquid chromatography and its application in analysis of protoberberine alkaloids in rat plasma [2]. In addition, we will present preparation of micropipette tip-based molecularly imprinted monolith for selective micro-solid phase extraction of berberine in plasma and urine samples [3].

References

Acknowledgements
This work was financially supported by NSFC (Nos 21375101,30973672, and 90817103) and the Fundamental Research Funds for the Central Universities.

Keywords: Liquid Chromatography, PAH, Plasma, SPME
Application Code: Bioanalytical
Methodology Code: Separation Sciences
Thin-layer chromatography (TLC) is a fast and inexpensive technique that is utilized for the separations of biomolecules; however, separation resolution on commercial TLC plates is compromised with increase in analyte size (molecular weight). This laboratory has recently employed capillary-channeled polymer (C-CP) films as a platform for protein separations and sample clean up from buffers and salt media prior to detection with mass spectrometry (MS). C-CP films have micron-sized channels that run the length of the film that induce spontaneous wicking of solutions. Aliquots (0.5-2 µL) of sample are loaded onto the C-CP film, and after addition of an organic mobile phase, proteins are displaced based on interactions with the mobile/stationary phases. After separation, the films are analyzed by matrix-assisted laser desorption/ionization (MALDI-MS) for identification of the separated proteins. Presented here are results illustrating how mobile phase composition, elution/loading volumes, and film length effect separation efficiency. The studied parameters are performed using 1 mL of a 2.5 M (each) ribosome, transferrin, and myoglobin suite in tris-HCl buffer. The optimal solvent composition of the matrix for these experiments was determined to be 0.1% trifluoroacetic acid, 80:20 ACN:H2O as it provides the most uniform and rapid crystallization of the sinapinic acid and the protein. The optimized parameters are employed on protein mixtures allowing for rapid high resolution separations with minimum sample and low solvent waste.

**Keywords:** Chromatography, Sample Preparation, Separation Sciences, Thin Layer Chromatography

**Application Code:** Bioanalytical

**Methodology Code:** Separation Sciences
Separation Sciences: Bioanalytical and Pharmaceutical

Toward Transmembrane Protein (TMP) -Functionalized, Biomimetic Stationary Phases for Ligand Screening

G-protein coupled receptors (GPCRs) and ion channels (ICs) are TMPs that respond to extracellular stimuli and regulate intracellular function; therefore, GPCRs and ICs constitute a large portion of known drug targets. The identification of ligands that regulate the activity of GPCRs and ICs is limited by the lack of reproducible, high-throughput screening methods. In this study, biomimetic stationary phases for high-throughput ligand screening were developed in both packed column and open-tubular separation formats. For packed column, 200 nm NH$_2$-labeled phospholipid vesicles were covalently attached to micrometer-sized, sulfonate-modified silica beads, forming silica core-vesicle shell particles. These particles served as packing materials for the column. In the open tubular format, phospholipid vesicles were attached to silica capillary walls by the same covalent linking chemistry. The vesicles were stabilized by introducing a polymerizable lipid, bis-Sorb PC, followed by UV-initiated crosslinking in the lamellar region of the lipid bilayer. The vesicles exhibited large surface area and reduced non-specific protein adsorption. The stability of the stationary phases was tested against physical and chemical insults commonly encountered in chromatography and capillary electrochromatography, such as high pressure, organic solvent, buffer changes and storage. TMPs were incorporated into the phospholipid vesicles and their stability and function were characterized. Affinity chromatography separations using the TMP-functionalized stationary phases will allow for high-throughput screening of TMP ligands. Compared to traditional cell-based screening assays, the controllable lipid and TMP composition, and stabilized phospholipid vesicle platform will lead to improved reproducibility. Funding was provided by NIH.

Keywords: Bioanalytical, Lipids, Membrane, Separation Sciences

Application Code: Bioanalytical

Methodology Code: Separation Sciences
One of the advantages of using Supercritical Fluid Chromatography for large scale separations is the ability to quickly separate chiral compounds with low solvent use and faster dry down times[1]. In the past many chiral/non-chiral presentations have been done, but typically with only a baseline separation being used as an example in the area of SFC. Contained in this study we have demonstrated Displacement separations and the issues involved. The body of work will include Retention Time RSD% and effects of slight co-solvent/modifier changes with regards to retention time correlated to the degree of displacement with comparison to recovery yield. Displacement chromatography has been discussed in the past [2] attempts at achieving practical true displacement SFC separation will be discussed.

INTRODUCTION
Chromatography is now a prominent tool at the forefront of chemical and biological developments to improve the human condition. Supercritical chromatography is recognized as a green technology impacting industries across the globe that enhances the preservation of natural resources, reduces waste generation and offers alternatives to fossil fuel-based infrastructures. Supercritical fluid chromatography (SFC) systems transforms CO₂ into a supercritical fluid. Originally developed in the 1960s, it regained popularity in the late 1990s. As a supercritical fluid, CO₂ demonstrates hydrocarbon-like properties under chromatographic conditions [4]. By substituting petroleum-based organic solvents with CO₂ in chromatography, greenhouse gas emissions and overall solvent pollution are significantly reduced. CO₂ is inert, safe and economical. Applications that have had a good record of success and visibility are chiral based separations that are used for large scale campaigns [5] and the throughput of SFC has been proven to be advantageous [6] elsewhere.

Keywords: Chiral, Chiral Separations, Chromatography, SFC
Application Code: Pharmaceutical
Methodology Code: Separation Sciences
To comply with FDA mandates on developing chiral drugs, the pharmaceutical industry often investigates enantiomeric separations early in the drug discovery process. In addition, chiral analysis is important in the production of specialty chemicals such as flavors, fragrances and pesticides (agrochemicals).

Developing chromatographic methods for chiral compounds is typically performed by either normal phase LC or supercritical fluid chromatography (SFC). In either case, stationary phases with chiral selectors are screened and the separation is optimized to separate the enantiomers. Due to the unpredictability of the separation, method development times can be time consuming and costly.

The use of analytical scale SFC will be presented for the development of chiral separations. The use of a low dispersion SFC instrumentation in combination with small particle (3 µm) columns packed in smaller diameter columns (3 mm) facilitates rapid development of chiral separations. We will present the critical factors for resolution of enantiomeric compounds, including different co-solvents and co-solvent mixtures, additives to the mobile phase, column length, temperature, and pressure. In addition, we will demonstrate the use of analytical SFC with multiple detection methods, most notably mass spectrometry, to further speed up method development times for chiral compounds. Applications such as in vivo stereoselective metabolism studies, analysis of chiral pesticide formulations, and chemical synthesis reaction monitoring will be highlighted.

Keywords: Chiral Separations, Pesticides, Pharmaceutical, SFC
Application Code: Pharmaceutical
Methodology Code: Separation Sciences
Capillary-channeled polymer (C-CP) fibers have been under investigation for several years as stationary phases for HPLC separation of macromolecules due to their extensive advantages over currently available phases. These shaped fibers are distinctive due to the eight capillary channels extending axially along the length of the fiber. These channels interdigitate when the fibers are packed into a column, to give open channels allowing for high linear velocities (>25 mm s⁻¹) and low backpressures (<2000 psi). The nonporous fiber surface allows for favorable mass transfer rates yielding efficient separations and modifications to the fiber surface chemistry. Reported here is the novel modification of polypropylene (PP) C-CP fibers with polyethylene glycol head group-modified phospholipids; termed PEG-lipids. Modification of the PP C-CP fiber surface with PEG-lipids proves a stable, robust, analyte specific surface without disrupting the physical integrity of the C-CP fiber. PEG-lipids contain a hydrophobic tail (aliphatic chain) that readily adsorbs to the PP surface, with the other end functionalized to include a head group providing specific binding capabilities for the target analytes. First, initial proof-of-concept studies evaluated the interaction of a biotin-PEG-lipid modified C-CP fiber columns with streptavidin labeled with Texas Red (SAv-TR). A TEM8 lysate modified with a mCitrine tag was spiked with SAv-TR and SAv-TR was captured from this mixture with little to no nonspecific binding. Second, a fluorescein isothiocyanate (FITC) modified PEG-lipid was studied as a function of concentration and linear velocity to yield excellent stability and breakthrough capabilities. With capacity and stability realized, is it evident that other functionalized PEG-lipids (maleimide, succinimidyl ester, amine, carboxylic acid) can be utilized with further modification to give a range of highly selective, analyte specific C-CP fiber stationary phases.
Separation Sciences: Bioanalytical and Pharmaceutical

Flow Rate Dependence on Chiral Selectivity and Resolution in SFC: Conventional Wisdom is Not Always the Best Advice

The Van Deemter equation describes the direct relationship between flow and chromatographic efficiency in liquid chromatography. This efficiency is directly related to resolution which is the main objective for any separation science. The conventional wisdom when using SFC is to use the highest flow rate possible due to the relatively flat VanDeemter curve. This mentality has been demonstrated by numerous publications and presentations. While this approach may work very well for achiral separations, the types of interactions that determine selectivity in chiral chromatography are very complicated, poorly understood and may require modification to this convention.

We have evaluated the effect flow rate has on the chiral resolution and selectivity of the enantiomeric pairs of several pharmaceutical compounds under typical SFC conditions. For all of our example compounds and an unmodified commercial SFC system, we found that a slower flow rate improves the resolution between the enantiomers. While the resolution dependence on flow rate is expected, selectivity is not known to be dependent on flow rate. When we evaluated selectivity for these compounds, for some but not all of the compounds, the selectivity improved at slower flow rates. The impact of flow rate on chiral SFC separations has not been well studied and these findings are interesting enough to suggest further research.

Keywords: Chiral Separations, Chromatography, Pharmaceutical, SFC
Application Code: Pharmaceutical
Methodology Code: Separation Sciences
Generic methods are commonly used for compound screening in high throughput and walk-up facilities. They are also used as the basis of many more refined methods. However, analysts occasionally encounter difficult separations for which selectivity is not easily obtained with a C18 column. These separations might require the use of more selective phases. Choices of bonding and end capping chemistry can vary selectivity. Retention factors are measured for neutral, acidic and basic compounds on modern columns including totally and superficially porous columns. Chromatographic conditions are varied including buffer/pH modifier, organic modifier (MeOH or MeCN), and column chemistry (C18, Polar Embedded, Phenyl-Hexyl, and other reversed and non reversed phase chemistries). These conditions are examined to determine selectivity differences and similarities across a range of commonly used LC/MS compatible conditions. The most orthogonal separation conditions are identified. The results of this will be compared to data from the hydrophobic subtraction model. Application of this information will be discussed in terms of one and two dimension separations.
Porous materials have gained recent interest due to their applications in DNA sequencing and tunable transport properties. Most studies have focused on either a single pore or a small array of cylindrical pores, which severely limit throughput and practical applications for lab-on-a-chip separations. In this work, we have explored nanoporous Au (NPG) as an alternative material in order to benefit from its high pore density. In addition, the random, bicontinuous geometry, inherent conductivity, and ease of surface modification of NPG enable unification of size- and charge-based separation techniques. Dynamic control over the separations process with the application of an external stimulus, such as an electric potential, is possible by tuning the surface properties of the NPG.

Herein, NPG was prepared by immersion of an Au-containing alloy in concentrated nitric acid, which preferentially dissolves less noble metals (e.g. Ni, Cu). Depending on de-alloying conditions or post-processing treatments, such as thermal annealing, pore sizes in the range of 30-80 nm have been obtained as verified under scanning electron microscopy. The NPG was sandwiched between two reservoirs for ionic and molecular transport measurements. Ionic transport was characterized by measuring the conductivity between two Ag/AgCl electrodes at various electrolyte concentrations. Sodium benzenesulfonate flux across the NPG was monitored via UV-Vis to observe rates of molecular transport as a response to different stimuli (e.g. electric fields, electrolyte concentration gradients). The Au surface was then modified following standard self-assembled monolayer (SAM) techniques with omega-functionalized thiols containing carboxylic acid, amine, or ferrocene moieties. As a result, molecular transport through the NPG was dictated by either solution pH or an electric potential applied to the NPG.

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: Electrode Surfaces, Materials Science, Nanotechnology, Separation Sciences
Application Code: Materials Science
Methodology Code: Separation Sciences
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<th>Session Title</th>
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<td>Abstract Title</td>
<td>Modification of Monolithic Structures with Carbon Based Nanoparticles for Liquid Chromatography</td>
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Abstract Text
The selectivity of carbon-based nanoparticles (CNPs) has attracted some interest in separation science. CNPs are being explored as potential stationary phases for chromatographic applications. We have explored the immobilization of CNPs on silica based monolithic columns; these include nanodiamonds (NDs) and carbon dots (C-dots). In this presentation, we focused on using C-dots as stationary phases for LC. The surface properties of the C-dots may provide interesting adsorptive characteristics as chromatographic materials, which have not been fully explored. C-dots were prepared by the decomposition of anhydrous citric acid in presence of 3-aminopropyltriethoxysilane (APTES) at 200 °C in glycerol. The resulting organosilane C-dot precursor was reacted with tetramethoxysilane (TMOS) by means of sol-gel processing to prepare monolithic columns. C-dots silica hybrid monoliths were successfully fabricated inside 50 [micro]m I.D. capillaries and as monolithic rods. Monolithic rods exhibit blue emission which suggests that the C-dot has been incorporated to the structure. The monolithic structure was preliminarily tested under different chromatographic conditions. The column preparation, spectroscopic characterization, and preliminary chromatographic evaluation will be discussed.

Keywords: Capillary LC, HPLC Columns, Liquid Chromatography, Modified Silica
Application Code: Other (Specify)
Methodology Code: Separation Sciences
Aptamers are single-stranded DNA or RNA which bind to various targets based on unique secondary and tertiary structures formed through changes in the oligonucleotide sequence. Aptamers have been developed against many targets including proteins, small molecules, and whole cells. Here we present the development of aptamers against intact mitochondria through the Systematic Evolution of Ligands through EXponential enrichment (SELEX) using magnetic immunoisolation techniques to separate bound from unbound fractions. This marks the first implementation of magnetic immunoisolation techniques used to develop aptamers against intact organelle surface proteins. Here, we first create an enriched mitochondrial fraction by incubating Anti-TOM22 Antibody coated magnetic beads to the whole cell lysate of 143B human osteosarcoma cells. This enriched mitochondrial fraction is then the target of subsequent rounds of aptamer development via SELEX. The relative binding of each ssDNA pool after each round of SELEX is characterized through capillary cytometry-LIF analysis, which is used to determine the selectivity of fluorescently labeled ssDNA (green) to bind to fluorescently labeled mitochondria (red). Thus far, seven rounds of aptamer development have been performed showing an increase in the selectivity of ssDNA pools relative to a naïve ssDNA library, ~80% selective binding of ssDNA to mitochondria compared to ~20% selective binding to the naïve library. Multiple sequences have been determined and synthesized indicating the presence of potentially interesting secondary structures. In future applications the potential aptamers may be used for many applications including mitochondrial labeling, enrichment of mitochondria from cell lysate, or the delivery of compounds to mitochondria surface targets.

**Keywords:** Bioanalytical, Nucleic Acids

**Application Code:** Other (Specify)

**Methodology Code:** Separation Sciences
The solution-cathode glow discharge (SCGD) is a source for elemental analysis of aqueous samples by atomic emission spectrometry. Unlike many atomic spectroscopy sources, it operates with relatively low power (around 70 W), can be sustained in atmospheric-pressure air, is small, and is inexpensive to construct. Detection limits for many elements are in the $10^{-9}$ g/mL range. Work will be presented on recent improvements in SCGD. These improvements include noise reduction made possible through use of a compact spectrograph, such as background correction and internal standardization. Enhancement of signals using low molecular weight organic compounds will also be discussed. The effects of these modifications on precision, accuracy, and detection limits will be presented.

**Keywords:** Atomic Emission Spectroscopy, Atomic Spectroscopy, Environmental/Water

**Application Code:** Environmental

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
Determination of Metal Concentrations in Nanocatalysts and in Metallo-Enzymes Using Microplasma-on-a-Chip Optical Emission Spectrometry

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.

But, 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 Pd in Pd-nanocatalysts encapsulated in arborescent graft polymers and the other of metals in a mettalo-enzyme.

**Keywords:** Atomic Spectroscopy, Lab-on-a-Chip/Microfluidics  
**Application Code:** Environmental  
**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
Trace metal analysis in pharmaceutical formulations is currently of great interest with the two new chapters of the USP (232 & 233) and the ICH Q3D regulations. The USP chapters identify ICP-OES and ICP-MS as techniques for the analysis of metals in pharmaceutical formulations. The accumulative metals of arsenic, cadmium, lead and mercury have been categorised as class 1 with the remaining metals as class 2,3.

This work considered the use of ICP-OES as a potential ‘walk-up’ technique and its application to both USP 232 & 233 and ICH Q3D requirements. A full optimisation of a Thermo iCAP 6500 Duo ICP-OES was carried out using both signal and signal to background ratio. The work looked at 71 wavelengths covering the 16 metals involved. From the optimum conditions LoD and LoQs were calculated for each of the elements which identified that ICP-OES was capable of analysing 12 of the required metals. This work then looked at using the NIST 3280 standard reference material, as a means for method validation. This is the only tableted SRM with certified values currently available. Using a CEM SP-D and a Milestone Ultrawave, as a preparative technique, the SRM was digested in nitric acid, reverse aqua regia and aqua regia at different temperatures. Samples were analysed by ICP-OES with the results being compared.

The NIST 3280 SRM has identified microwave digestion as an excellent technique for tablet digestion. The ICP-OES has demonstrated that it can achieve high percentage recovery, 95-105% in the SRM.

It was identified that the certified values of the class 1 metals were too low to be detected by OES after dilutions were applied for sample analysis. A Thermo X Series ICP-MS was considered for the determination of the 4 class 1 metals As, Cd, Hg and Pb, as the additional sensitivity gained from MS was considered to be necessary for this application.


**Keywords:** ICP, ICP-MS, Pharmaceutical, Plasma Emission (ICP/MIP/DCP/etc.)

**Application Code:** Pharmaceutical

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
Previous work demonstrated the utility of the evaporative technique when used with Concentrated Multiple Reflection ATR (CMRATR) spectroscopy. A droplet (5 to 20 microliters) of a solution with a volatile solvent is placed on the sampling surface of a CMRATR accessory. When the solvent evaporates, the solute is left in intimate contact with the ATR sampling surface. The spectrum, free of solvent, can then be taken with the high sensitivity inherent in CMRATR. The combination of technique and equipment is directly applicable to proteomic, forensic, archaeological, and pesticide analyses. It was shown that the technique can be used for quantitative as well as qualitative analysis. The data taken were based on organic dyes dissolved in volatile organic solvents with evaporation times under eight minutes. Difficulties were encountered, however, when less volatile aqueous solutions were attempted. Evaporation times were greater than one hour and low sensitivity was obtained, perhaps due to greater ring staining outside the active area of the ATR sampling surface. The application to water samples is necessary, however, to make the technique/equipment "universal."

Increasing the rate of evaporation may suppress the ring staining effect.

Two different approaches will be explored for accelerated evaporation, with the impact on sensitivity and precision studied. In the first, two different organic solvents having different volatilities will be used with the same solute and solute concentration. In the second, the temperature of the sampling surface will be adjusted to two different elevated temperatures to determine the effect on the same aqueous solution.

Keywords: Environmental Analysis, Forensics, FTIR, Proteomics
Application Code: General Interest
Methodology Code: Vibrational Spectroscopy
New Developments in Analytical Instrumentation and Software

Saliva as a Matrix for Establishing the Exposure of Drugs as Alternative to Plasma Using MEPS as Sampling Technique

Blood, plasma and urine matrices are traditionally used in pharmacological studies of drugs in clinical and preclinical phases. In addition drug metabolism and pharmacokinetic applications, for example, metabolite identification, are often run using plasma as the matrix. The use of alternatives to blood and plasma for establishing exposure to drugs has become a significant direction in clinical and forensic toxicology. The choice of saliva vs blood or plasma should be addressed seriously, given the well-recognized fact that saliva offers rapid and non-invasive sampling.

In this work microextraction by packed sorbent (MEPS) was used to collect exact sample volume and for sample preparation and lidocaine used as model substance. We have demonstrated the use of MEPS technique with LC-MS/MS as a tool for the screening and determination of lidocaine in saliva samples. MEPS technique provides speed and simplicity of the sample-preparation process. Furthermore, the present method reduces the sample preparation time (<1min per sample).

Keywords: Sample Handling/Automation, Sample Preparation, Separation Sciences
Application Code: Biomedical
Methodology Code: Sampling and Sample Preparation

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New Developments in Analytical Instrumentation and Software

Challenging GC-MS Applications Achieved with Cold El

Cold El is electron ionization of cold molecules in a supersonic molecular beam (SMB). In GC-MS with Cold El the GC is interfaced with the MS using an SMB and electron ionization is performed in a fly-through ion source. GC eluting compounds are mixed with helium, expand from a supersonic nozzle into a vacuum chamber, vibrationally cooled, skimmed, and pass a fly-through EI ion source where they are ionized by 70 eV electrons and mass analyzed.

GC-MS with Cold El enables the analysis of samples that otherwise would be hard or impossible to deal with. Recent challenging applications that were explored using a 5975-SMB GC-MS with Cold El include:

A) Pulsed flow modulation GCxGC-MS with cold El for the combination of improved separation and identification.
B) Open Probe fast GC-MS with Cold El for the provision of real time analysis with separation.
C) Improved sample identification was achieved via isotope abundance analysis software that provides elemental formulae and is automatically linked with the NIST library.
D) Isomer distribution analysis method was developed for fuel and oil characterization.
E) Ants way of communication was elucidated based on their wax isomers barcode.
F) Low levels impurities were analyzed in active pharmaceutical ingredients.
G) Chemical reaction products were analyzed for the optimization of chemical reaction yields.
H) A range of advanced forensic applications were investigated.

These examples will be shown to demonstrate improved MS information, extended range of compounds amenable for analysis, faster analysis and improved sensitivity provided by Cold El.

Keywords: Gas Chromatography/Mass Spectrometry, Instrumentation, Mass Spectrometry

Application Code: General Interest

Methodology Code: Gas Chromatography/Mass Spectrometry
Immediately after Superstorm Sandy hit the east coast in October 2012, the project titled SUDS (Send Us your Dirt from Sandy) was launched. The objective of this research was to reach out to citizens living in the affected areas to obtain soil samples rapidly. Using social networking and the media, large amounts of samples were collected in a short period of time. Over 60 samples from various areas around Manhattan, Queens and Brooklyn were collected and tested for organic and metallic contaminants. The samples were prepared for the organic analysis using microwave assisted extraction. Each sample was then tested for polychlorinated biphenyls (PCBs), using gas chromatography combined with electron capture detection (GC-ECD), and polycyclic aromatic hydrocarbons (PAHs), using gas chromatography combined with mass spectrometry (GC-MS). Detectable concentrations of PCBs and PAHs were found along the Rockaway Peninsula, Newtown Creek and the Gowanus Canal. For the metallic analysis, samples were dried, ground and tested for lead and arsenic using X-ray fluorescence spectrometry (XRF). High concentrations of arsenic were found along the Rockaway Peninsula, Newtown Creek and the Gowanus Canal. High concentrations of lead were found only in the Newtown Creek area. To determine the effects of renovation and rebuilding, additional samples were obtained in July 2013 from the Rockaway Peninsula and the Newtown Creek areas and measured for lead and PAH concentrations.

Keywords: Metals, PAH, PCB’s, Soil
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
More than 10 kinds of core shell particle column have been available recently because core shell media offers significant improvements such as higher efficiency and lower pressure drop for existing HPLC operations without having to replace existing HPLC systems with UHPLC systems. Moreover, large molecules such peptides or proteins have been watched as medicine in recent years. Silica gel with 30 nm pores has generally been used for separation of proteins.

In this study, a 2.6 \( \text{mm} \) core-shell silica with a non-porous core approximately 1.6 \( \text{mm} \) in diameter and a superficially porous layer of 0.5 \( \text{mm} \) and 30nm pore was developed. As a novel bonding chemistry, hexa-functional C18 reagent with two sets of trichlorosilane was applied. It is considered that this reagent makes acidic stability high because of six positions of siloxane at most. Acidic stability was evaluated under 0.1 % formic acid solution/acetonitrile as a typical LC/MS mobile phase condition at 70 degree Celsius. It was confirmed that the hexa-functional C18 was stable for more than 1000 hours. The developed materials bonded with hexa-functional C18, C8 and C4 were evaluated to separate not only standards of peptides and proteins but also tryptic digest of a protein using UV and Mass spectrometry detectors.

Keywords: HPLC, Peptides, Protein
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
### Abstract Text

Catalytic Combustion Ionization Detection (CCID) is a GC method in which the sample compound itself fuels a momentary burst of flame ionization as it impacts a catalytically active ceramic surface in a gas environment containing Oxygen. The process selectively ionizes compounds containing chains of Methylene (CH2) groups while providing negligible response to Aromatic or Cyclo-Hydrocarbons. By judicious control of the concentration of Oxygen in the detector gases, additional selectivity is achieved for Linear versus Branched Hydrocarbons, and Hydrocarbons with Saturated versus Unsaturated Carbon bonds. When applied to analyses of complex petroleum samples, CCID simplifies the chromatographic fingerprint by eliminating Aromatic and Cyclo-Hydrocarbon peaks, and revealing only Alkane and Alkene peaks. CCID chromatograms of Gasoline, Auto Diesel Fuel, Fuel Oil, Kerosene, Biodiesel, and Reformate and Naphtha standards illustrate the unique selective analyses possible by this detection technology.

**Keywords:** Detector, Fuels\Energy\Petrochemical, Gas Chromatography, GC Detectors

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

**Methodology Code:** Gas Chromatography
The leaves of Gymnema sylvestre have been chewed for over a century as a folk remedy for diabetes because of its ability to block sweet taste receptors. The polypeptide responsible for this effect in rats is called gurmarin.

The isolation of gurmarin from Gymnema sylvestre is a long process which includes a water extraction, an acid precipitation, an ethanol extraction, an ion chromatographic separation, and purification by high performance liquid chromatography. The published process is expected to have a 0.001% yield. Our research aim is twofold: to improve this yield by investigating and optimizing a method published by other researchers and to isolate and purify a sufficient quantity of gurmarin so that it can be used for in vivo medical research studies.

The preliminary results indicate that the elimination of a preparative column purification step in the published procedure can save considerable time and still provide adequate purification. We have also used synchronous florescence spectra to identify the gurmarin fractions by their tryptophan and tyrosine peaks.

**Keywords:** Bioanalytical, HPLC, Isolation/Purification, Peptides

**Application Code:** Bioanalytical

**Methodology Code:** Liquid Chromatography
New Developments in Analytical Instrumentation and Software

The Determination of Acrylamide in Fried Potato Crisps by Solid Phase Extraction

Studies have shown that acrylamide is a kind of carcinogen presenting in fried food, such as fried potato crisps. The analysis of acrylamide content was carried out by using High Performance Liquid Chromatography. The acrylamide in potato chips was extracted by water, then removed fat with hexane by liquid-liquid extraction. Discarded the upper layer of hexane after centrifugation, then extracted acrylamide from the rest of water by solid-phase extraction method with a kind of carbon which was designed to be Cleanert ACA (500mg/12mL). The method for determination of acrylamide is good for its stability and sensibility, The detection limit is 0.5\(\text{g/mL}\) and average recovery is about 85%. It can quickly and effectively detect acrylamide in potato chips.

Keywords: Sampling, Solid Phase Extraction
Application Code: Food Science
Methodology Code: Sampling and Sample Preparation
Garlic (Allium sativum) cloves were crushed in a special press with a piston and cylinder. After crushing, the piston was removed and parafilm stretched over the cylinder. The solid phase microextraction (SPME) needle was pushed through the parafilm and the semi-polar SPME fiber was then exposed to the vapor above the crushed tissue for 5.0 minutes. The SPME fiber was then analyzed using a gas chromatograph-mass spectrometer (GCMS). The injector temperature was critical in the analysis. At low injector temperatures the chemicals in the SPME did not properly desorb. At high injector temperatures the chemicals decomposed. The column temperature was increased from 60°C to 120°C at 5°C/min. The transfer line temperature was 280°C.

Some of the major chemicals found in garlic vapor are: methyl-2-propenyl disulfide, methyl propyl disulfide, di-2-propenyldisulfide, propyl-2-propenyl disulfide and dipropyl disulfide. It is believed that several of the disulfides were formed from the decomposition of thiosulfinates in the injection port of the GC.

Several different types of garlic (Allium sativum) and elephant garlic (Allium ampeloprasum) were analyzed using the new method. The method could distinguish the different varieties from one another from the distribution of the chemicals observed.

Keywords: Agricultural, Food Science, GC-MS
Application Code: Food Science
Methodology Code: Gas Chromatography/Mass Spectrometry
Essential oils were extracted from 3.0 grams of either Osage Orange leaves or fruit using ~ 50 mL of methylene chloride with an internal standard, m-xylene, for a period of four weeks. The extract was then concentrated to ~2.0 mL by boiling off the solvent and analyzed by gas chromatography-mass spectrometry (GCMS).

About four oils were found in the leaves and these have not yet been identified. They appear to be high molecular weight n-alkanes. Their concentration in the leaves was quite small. It was found that only 0.03 +/- 0.01 % of the leaf mass was soluble in methylene chloride.

Approximately eight oils were found in the fruit, but have not yet been identified. However, their structure is more complex than simple n-alkanes with a number of phenolic compounds observed from the mass spectra. The concentration of oils found in the fruit is much larger than that found in the leaves. Approximately 0.28 +/- 0.20 % of the fruit mass was soluble in methylene chloride.

Keywords: Environmental, Extraction, GC-MS
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
An initial study demonstrated the feasibility of a unique FTIR liquid cell. When used in conjunction with a variable angle reflection accessory, ATR spectra could be taken at angles above the critical angle. At an angle under critical, light passed through the sample, reflected from the bottom of the cell, and a double-pass transmission spectrum was recorded. Above the critical angle, the ATR angle could be adjusted from grazing to near critical. Below the critical angle, a significant increase in sensitivity was observed.

There were some difficulties. For transmission spectra, a KBR plane window was substituted for the ATR hemisphere. The pathlength provided for external reflection was fixed and excessive. Low optical throughput was obtained in the transmission mode due to the poor finish of the liquid cell bottom.

With the new cell, reported here, the same hemisphere will serve as the ATR element and the transmission window. The transmission pathlength will be alterable, within limits, by changing a spacer under the hemisphere. The spacers will be chosen to provide pathlengths of more modest dimensions. To answer the throughput problem, two subsequent studies were conducted to determine the best finishing techniques for 316 stainless steel and for Hastelloy B-3 alloy. It was found that traditional optical polishing produced the best results in terms of reflectivity, flat response, and reproducibility and this process will be used for the new cell.

**Keywords:** FTIR, Instrumentation, Spectroscopy, Vibrational Spectroscopy

**Application Code:** General Interest

**Methodology Code:** Vibrational Spectroscopy
Essential oils were extracted from 3.0 grams of cedar leaves using ~ 50 mL of methylene chloride with an internal standard, m-xylene, for a period of four weeks. The extract is then concentrated to ~2.0 mL by boiling off the solvent and analyzed by gas chromatography-mass spectrometry (GCMS).

Some of the major essential oils found in cedar leaves are: -pinene, limonene, safrole and cuparene. By far the compound in greatest abundance was sabinene, comprising 27 +/- 5% of the total essential oils. Essential oils comprised 0.8 +/- 0.2 % of the mass of the cedar leaves.

Samples were collected monthly for two years. It was found that during the hot and dry periods of the year the concentration of essential oils quadrupled compared to the concentration in the summer. The distribution of essential oils shifted to favor higher molecular weight compounds during the summer months. The heat and drought of the summer are at least partly responsible for the change in distribution of essential oils.

Keywords: Environmental, GC-MS
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Essential oils were extracted from 3.0 grams of pine leaves using ~50 mL of methylene chloride with an internal standard, m-xylene, for a period of four weeks. The extract is then concentrated to ~2.0 mL by boiling off the solvent and analyzed by gas chromatography-mass spectrometry (GCMS).

Some of the major essential oils found in pine leaves are: alpha-pinene, beta-pinene, limonene, beta-myrcene, camphene and oximene. By far the compound in greatest abundance was alpha-pinene, comprising 43 +/- 18% of the total essential oils. beta-pinene was the second most abundant comprising 17 +/- 13% of the total essential oils. Essential oils comprised 0.33 +/- 0.20 % of the mass of the pine leaves.

Samples were collected monthly for a period of ten months. It was found that during the hot and dry periods of the year the concentration of essential oils was several times greater than that in the winter. Also, the distribution of essential oils shifted to favor higher molecular weight compounds during the summer months.

Keywords: Environmental, Extraction, GC-MS
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Onion (Allium cepa) tissue was crushed in a special press and the juice was collected in a test tube. A semi-polar solid phase microextraction (SPME) fiber was then exposed to the vapor above the juice for 5.0 minutes. The SPME fiber was then analyzed using a gas chromatograph-mass spectrometer (GCMS). The injector temperature was critical in the analysis. At low injector temperatures the chemicals in the SPME did not properly desorb. At high injector temperatures the chemicals decomposed. The column temperature was increased from 60°C to 120°C at 5°C/min. The transfer line temperature was held constant at 280°C.

Some of the major chemicals found in onion vapor are: \( \text{z-propanethiol-(S)-oxide} \), 2-methyl-2-pentenal, methyl propyl disulfide, methyl-1-propenyl disulfide, dipropyl disulfide, propyl-1-propenyl disulfide and di-1-propenyldisulfide. We hypothesize that several of the disulfides were formed from the decomposition of thiosulfinates.

Four different types of onions were analyzed using the new method. Although the chemicals observed by GCMS are believed to be decomposition products the different types of onions could be distinguished from one another from the distribution of the chemicals observed.

**Keywords:** Agricultural, Food Science, GC-MS

**Application Code:** Food Science

**Methodology Code:** Gas Chromatography/Mass Spectrometry
In this poster we discuss the aspects of a new core-shell product that offers the analyst speed of analysis, increased sensitivity and lower backpressure than previously available. We discuss how is core-shell technology is improving the productivity of the analyst whilst utilising traditional HPLC systems. There is still a lot to learn from a manufacturing point of view with core-shell technology and we highlight how one core-shell can be very different from another. Van deemter equations provide some of the answers but not all of the variables are covered, so we look at the comparisons possible.

How does this technology differ from the use of UHPLC and which one is the correct choice in any given situation? Given that both offer high speed, high sensitivity? We look at questions such as this and try to point out the benefits and weaknesses that each technology may possess.

Keywords: Chromatography, High Throughput Chemical Analysis, Liquid Chromatography, Modified Silica
Application Code: General Interest
Methodology Code: Separation Sciences
Much has been made of the ability of core-shell particles to improve speed of analysis and sensitivity. In this poster we look at the role that resolution plays when trying to improve speed of analysis, as we go quicker and quicker selectivity (alpha) plays a large role in the separation process. We look at the alternative phases that are available on core-shell technology and how these can impact how fast we can actually run.

What selectivity do we actually require in order to develop a new method? Orthogonality of the stationary phase being paramount in the separation of closely related species, metabolites and isomers. The choice of stationary phase will also impact on our productivity in terms of the lifetime and stability of the analysis along with the reproducibility that is acquired.

Keywords: HPLC, Liquid Chromatography, Liquid Chromatography/Mass Spectroscopy, Pharmaceutical

Application Code: Pharmaceutical

Methodology Code: Liquid Chromatography
Bisphenol A is widely used in plastic and other industrial consumer products. Release of bisphenol A and its analogues into the aquatic environment during manufacture, use and disposal has been a great scientific and public concern due to their toxicity and endocrine disrupting effect on aquatic wildlife and even human beings (Zhu and Zuo, 2013, Adv. Environ. Res. 2, 179-202). More recent studies have shown that these alkylphenols may affect the molting processes and survival of crustacean species such as American lobster and shrimps. In this study, we have developed GC-FID and GC-MS methods for the determination of bisphenol A and its analogues in shrimp and lobster samples. Bisphenol A, 2,4-bis-(dimethylbenzyl)phenol and 4-cumylphenol were found in shrimp Macrobrachium rosenbergii in the concentration range of 0.67–5.51, 0.36–1.61, and 1.96 ng/g (wet weight), respectively. In lobster tissue samples, bisphenol A, 2-t-butyl-4-(dimethylbenzyl)phenol, 2,6-bis-(t-butyl)-4-(dimethylbenzyl)phenol, 2,4-bis-(dimethylbenzyl)phenol, 2,4-bis-(dimethylbenzyl)-6-t-butylphenol and 4-cumylphenol were determined at the concentration range of 4.48-29.2, 0.90-4.62, 2.71-28.1, 0.35-3.66, 0.64-4.76, and 0.44-5.94 ng/g (wet weight), respectively. The effects of these endocrine disrupting alkylphenols on the molting of shellfishes will be reported at the presentation.

Keywords: Biological Samples, Environmental Analysis, Environmental/Biological Samples, Gas Chromatography
Application Code: Bioanalytical
Methodology Code: Gas Chromatography/Mass Spectrometry
Determining the Provenance of Albanian Artifacts Using Solution-Based ICP-MS and Laser-Ablation ICP-MS

The knowledge of the provenance of artifacts can help in determining trade routes. To determine the provenance of artifacts collected in Albania, ICP-MS and LA-ICP-MS were used for trace metal analysis. In this study, pottery samples collected from different regions in Albania were analyzed by trace metal analysis. The samples were prepared for solution-based ICP-MS analysis using microwave digestion. The microwave digestion procedure consists of dissolving the samples in hydrofluoric and nitric acid and heating the samples at elevated temperatures and high pressures to release metals for analysis by ICP-MS. Samples from different regions were compared in terms of mineral content to determine their provenance. Since different geographic regions would usually differ in mineral content, the information obtained by comparing samples from different regions can help in the determination of trade routes.

Abstract Text

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Keywords: Art/Archaeology, ICP-MS, Laser
Application Code: Art/Archaeology
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Methylxanthines can serve as biomarkers for certain compounds and can be analyzed using high-performance liquid chromatography – mass spectrometry to determine the presence of chocolate, as well as various herbs and elixirs. For example, cacao (chocolate) has a unique chemical composition which consists of hundreds of different compounds. However, since cacao is one of the few plants that contain theobromine as the primary methylxanthine, theobromine can be used as a marker for the presence of cacao. In this study to determine whether cacao (chocolate) was consumed in vessels, we analyzed pottery for methylxanthine compounds, primarily theobromine and caffeine. Analysis of other relevant bio-markers such as theophylline to distinguish between cacao use and other elixirs will be discussed.

Keywords: Art/Archaeology, HPLC, Liquid Chromatography, Mass Spectrometry
Application Code: Art/Archaeology
Methodology Code: Liquid Chromatography/Mass Spectrometry
We describe the extension of photomicroscopy and digital imaging to the determination of heterogeneous rate constants and the energy of activation of indium mediated allylation (IMA). IMA is a relatively new tool for synthetic chemists and represents Green chemistry as the reaction proceeds rapidly and with excellent yield in water. Solvent effects for this reaction are dramatic, so we study the rate of reaction in a variety of solvents. Furthermore, we have prepared an Arrhenius plot in order to estimate the energy of activation. The applicability of photomicroscopy to the study of heterogeneous reaction rate is demonstrated. Precision and accuracy are discussed. This material is based upon work supported by the National Science Foundation under Grant No. CHE-1007510.

Keywords: Electrochemistry, Imaging, Microscopy, Surface Analysis
Application Code: Other (Specify)
Methodology Code: Microscopy
New Developments in Analytical Instrumentation and Software

Air Pollution Observations in Chicago from 2002-2012

During two consecutive 3-year summer periods between 2002 and 2012, studies on air pollution in Chicago, Illinois were completed. Meteorology and trace gas mixing ratios were recorded concurrently while two aerosol samples (segment A and B) were collected each day during all summers’ studies. The air sampling campaigns were completed to determine how local meteorology and lake breezes influence the concentrations of trace gases and secondary air pollutants. Presenting results from the most recent study in summers 2010, 2011, and 2012 and comparing meteorology and pollution concentrations to the preceding 3-summer studies in 2002–2004 is the main focus of this paper.

Each sample collection day was classified as reference, lake breeze, or variable depending on the recorded wind direction during aerosol sampling segments. Reference days of steady wind direction were the most commonly observed day classification in both 3-summer studies. Southwesterly winds dominated reference days in summers 2002, 2003, and 2010 while westerly winds were predominant in 2004 and 2012. Northerly winds were often recorded in summer 2011 collections. Half of the number of lake breezes were observed in summers 2010–2012 compared to 2002–2004 and the observation of variable days slightly increased in the former study. Unpooled, two independent sample t-tests showed that in summers 2002–2004 average ammonium concentrations were the only pollutant significantly affect by a lake breeze when compared to non-lake breeze day average concentrations. In summers 2010–2012, chloride, nitrate, and sulfate ion concentrations in addition to ozone mixing ratios were also significantly different at 95.0% confidence level compared to averages on non-lake breeze days. Overall, with respect to trace gases, average ozone mixing ratios increased by 18.2% from summers 2002–2004 to 2010–2012 whereas mixing ratios of nitrogen oxides decreased by 49.0%. The concentration of various water-soluble ions varied in each collection campaigns. Both average sulfate and chloride ion concentrations decreased from 2002 to 2012, 5.2% and 26.8%, respectively. A large increase in the average concentration of calcium (171%) and oxalate (53.3%) was found when comparing 2002-2004 to 2010-2012 data. Phosphate and nitrate averages also increased, 14.1% and 7.7%, respectively. Humidity and temperature averages increased from the earlier to most recent studies, however, wind speed averages decreased.

Keywords: Environmental, Environmental/Air, Ion Chromatography
Application Code: Environmental
Methodology Code: Physical Measurements
Glycosaminoglycans (GAGs) are linear polysaccharides consisting of repetitive disaccharide units of aminosugars alternating with an uronic sugar or galactose. The family of GAGs includes several compounds such as heparin (HEP), chondroitin sulfate (CS), dermatan sulfate (DS), and hyaluronic acid (HA). HEP has been widely used as an anticoagulant and quality control of HEP is most important due to this widespread medical use and a previous tragic case of adulteration, ascribed to oversulfated chondroitin sulfate (OSCS). The current USP method for quality control of heparin is based on ion exchange chromatography (IEC) and UV detection. In spite of the ability to separate heparin from OSCS, this method has a long total run time, estimated to be 75 min, and drift in the baseline is problematic. Alternatively, a preliminary ion exclusion liquid chromatography (IELC) separation of HA, HEP and OSCS was first attained by us in 6 min using a polymethylmethacrylate-based strongly cation exchange resin (particle size 10 μm) by using 1 mM phosphoric acid (pH 3.2) at a flow rate of 0.5 mL/min with UV detection at 210 nm. The elution order seemed to be related to the number of sulfate groups in the compounds. HA, which does not have any sulfate groups, was the least retained GAG. In HEP, there are two sulfate groups per monomer while the degree of sulfonation in OSCS can definitely be higher than HEP. Professional optimization software was used to simultaneously vary four different variables: ionic strength, pH, flow rate, and sample injection volume. These parameters were optimized based on the four responses: the tailing of the HA peak, resolution of HA from HEP, resolution of HEP from OSCS, and run time. The best IELC separation using the same column was achieved with a mobile phase consisting of 0.01 M phosphoric acid, pH 2.3 at a flow rate of 1 mL/min and injection volume of 25 μL. The method provides a fast indication in 4 min of potential HEP contamination by OSCS.
Recently, we have published ESI and MALDI data that employed one induction based fluidic source/dispensing tool for BOTH 100% efficient, millisecond ESI sample introduction and MALDI sample placement. This June 2013 Analytical Chemistry paper and others published in JASMS, Polymer, and RCM over the last five years addressing MALDI demonstrated, the fastest MS sample introduction and sample placement tool on the planet for ESI sample introduction and MALDI deposition.

In this poster, we demonstrate that ONE DEVICE, the nanoLiter Cool Wave, can be used to directly launch 100% of samples containing proteins, peptides, drugs of abuse, metals (alkali, transitions, lanthanides), and mixtures; such as, coffee, water, urine, whole blood and other samples near/into ESI ToF/MS systems in one case generating 6400 determinations in one day, showing video thereof. We also present data showing that the same device can dispense nanoLiters and make excellent MALDI crystals that literally yield a 10 to 100x improvement in sensitivity compared to microliter sized samples run under identical conditions.

Finally, we discuss the physics of sprays and IBF which shoot drops like this....................... in a straight line, as we explain why spraying is electrostatically ignorant.
Submicrometer particles improve separation efficiency by virtue of the shorter distances for mass transport, which is particularly valuable for large proteins, such as monoclonal antibodies. In reversed-phase liquid chromatography, the onset of slip flow from the nanoscale dimensions among submicrometer particles enables the use of existing UHPLC instrumentation to achieve optimal flow rates for these separations. Minimizing deleterious processes for therapeutic monoclonal antibodies, including aggregation and disulfide scrambling, is important to characterize to minimize their occurrence, yet these species have not previously been sufficiently resolved. We study the extent of improvement with submicrometer particles in silica capillaries, and we extend these studies to submicrometer particles in stainless steel columns.
Despite widespread use throughout the pharmaceutical industry, chromatographic protein purification is not completely understood at the molecular-scale. We report direct measurement of super-resolved single-ß-lactalbumin adsorption/desorption at single ligands on an agarose support, allowing the first direct test of a molecular-scale statistical theory of protein adsorption that was developed over 50 years ago. The combination of super-resolution experiment, kinetic analysis, and statistical theory reveal that, although industrial ion-exchange separations commonly employ single-charged ligands for ion-exchange adsorption, at the molecular scale this process is entirely driven by accidental ligand clustering. Together with a molecular-scale comparison of engineered vs. accidentally clustered ligands, the resulting simulated chromatographic elution profiles demonstrate that engineered ligand clusters should be pursued for next-generation stationary phases for ion-exchange protein purification.

Keywords: Bioanalytical, Ion Exchange, Spectroscopy, Statistical Data Analysis
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
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**Abstract Text**

The column has always played a pivotal role in the capability of HPLC to solve “real world” problems. From the early days of large particle superficially porous packings (SPP) to decades of domination of 5-μm reversed-phase columns to today’s modern monolithic, sub-two micron porous and the (revisited) small particle SPPs, the state of column technology has often led and sometimes fallen behind the capabilities of the instrumentation that it supports. This presentation will focus on the more recent developments in HPLC (and the newer term UHPLC) columns that have brought liquid phase separations to a high level of contribution to the analytical laboratory. Today’s columns provide efficiencies of hundreds of thousands of theoretical plates but the smaller particles give rise to increased back pressure which causes problems with some of the physiochemical parameters that govern separations. After a brief look at the history of particle development, I will spend the majority of my time focusing on the latest stationary phase materials: sub-two micron porous particles, SPP and monoliths (silica- and polymer-based). Without getting too involved in the theory, I will discuss the pros and cons of these columns and give some practical advice on which approach might fit better into a typical chromatography laboratory. Getting these columns to work with modern HPLC/UHPLC instrumentation at their optimum performance has brought a great deal of attention on instrumental band dispersion, uneven frictional heating, pressure-retention relationships and other experimental effects that weren’t evident at sub-400 bar pressures of traditional HPLC.

**Keywords:** Chromatography, HPLC

**Application Code:** Other (Specify)

**Methodology Code:** Liquid Chromatography
For analysis of complex samples, the need for higher resolution separations requires an understanding of the timescale of processes responsible for analyte retention in the stationary phase. In this work, we build upon the pioneering work of Mary J. Wirth and her students, to probe dynamics of molecules interacting with reversed-phase chromatographic materials. Single-molecule fluorescence imaging is used to observe transport of individual molecules within RPLC silica particles, a technique that allows direct measurement of molecular residence times, diffusion rates, and the spatial distribution of molecules within the particle. Based on the localization uncertainty and characteristic measured diffusion rates, statistical criteria are developed to resolve frame-to-frame behavior of molecules into moving and stuck events. The measured diffusion coefficient of moving molecules is used in a Monte-Carlo simulation of a random-walk model within the cylindrical geometry of the particle diameter and microscope depth-of-field. The simulations are in good agreement with experimental data, indicating transport of moving molecules in the porous particle is described by a random-walk. Histograms of stuck-molecule event times, locations, and their contributions to intraparticle residence times are also determined. We are also developing a method to observe intra-particle diffusion on a 30-times faster timescale by combining fluorescence correlation spectroscopy (FCS) with single-molecule imaging. We image a small region on the CCD camera that can be framed at millisecond rates, where autocorrelation of intensity from this region yields molecular diffusion rates. We control acquisition electronically by choice of pixels on the camera to manipulate location and size of the active area, so that the variation in the autocorrelation relaxation with mean-square displacement can be measured in order determine the intraparticle diffusion coefficient.
Post-translational modifications (PTMs) to histone proteins constitute a major type of epigenetic mechanism that regulates chromatin structure and gene expression patterns in eukaryotes. In addition to their important roles in standard physiology, disruptions in histone PTM signaling patterns have been suggested to be significant, potentially causative factors in various human diseases such as cancer. As most histone PTM work in the chromatin biology field is accomplished using site-specific antibodies, the quantitative measurement of the dynamics of histone PTMs been largely unmet as all current approaches view histone PTMs as a static event. We have specifically addressed this deficiency by developing novel mass spectrometry based proteomic methods to quantitatively investigate the kinetic/dynamics of histone methylation, acetylation and phosphorylation using metabolic in vivo labeling strategies. These studies in combination with biological experiments will help provide a systems biology outlook on gene expression that will lay down the basic scientific foundation to advance several applications, such as stem cell reprogramming and cancer progression.
Lung cancer is the leading cause of cancer-related death in the USA and worldwide. Due to the late stage of diagnosis, poor efficacy of chemotherapies, and development of drug resistance, the 5-year survival rate is 16%. Genomic sequencing has revealed mutations in human lung cancers, e.g. EGFR, KRAS, ALK, and RET, that control oncogenic processes including evasion of apoptosis, cellular proliferation and cellular invasion. Unfortunately, targeted therapies for these kinases ultimately fail due to acquired resistance. To identify additional targets for treatment inherently transparent to genomic sequencing, new technologies must be developed, implemented and validated at the level of proteins and post-translational modifications. Quantitative proteomics and phosphoproteomics are poised to improve our understanding of oncogenic signaling. We implemented a quantitative phosphoproteomic strategy to identify mitotic substrates of two families of mitotic kinases, Polo-like kinases and Aurora kinases, which are amplified or overexpressed in lung cancer. We increased the number of known phosphorylation loci attributed to Polo-like kinase 1, Aurora A, and Aurora B by 10-fold. We then implemented a robust and accurate quantitative phosphoproteomics strategy to interrogate the global phosphorylation profile of primary human lung tumors. We quantified ~9000 phosphorylation sites across signaling pathways including KRAS, PI3K, and ERBB2, and identified the enrichment of PLK1-specific substrates in one of these human tumors. To ensure that the proteome and phosphoproteome of patient tumors was stable during processing by pathologists, we quantified protein and phosphopeptide abundances after tumor resection. Having established a robust analytical workflow for kinase network profiling by quantitative phosphoproteomics, it should now be possible to identify patient-specific phosphoproteomic profiles to aid informed decision making for personal treatment strategies.

Keywords: Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry, Protein, Proteomics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Mass Spectrometry
Recent advances in instrumentation and experimental design have propelled mass spectrometry to the forefront for protein identification, biomarker discovery, and quantitative proteomics. Conventional bottom-up proteome analysis is based on the ability to sequence the constituent peptides of an enzymatically digested protein mixture. This limits the ability to determine sequence truncations, single nucleotide polymorphisms, and the combinatorial nature of PTMs. The top-down approach, an alternative that involves the MS/MS analysis of intact proteins, provides both intact protein and fragment mass measurements and yields unparalleled maps of sequence and modifications. Activation and dissociation of intact proteins remains one of the major hurdles which has limited the widespread adoption of the top-down approach for proteomic studies. We have recently implemented ultraviolet photodissociation (UVPD) on a high performance mass spectrometer (the Orbitrap platform). UVPD affords broader sequence coverage via more extensive backbone fragmentation than can be obtained using any other MS/MS method, and ion activation/dissociation can be accomplished using a single 5 nanosecond laser pulse. This translates to an MS/MS technology that can characterize intact proteins in detail, including mapping post-translational modifications (PTMs) such as phosphorylation that play critical roles in cell signaling and metabolic regulation.
Abstract Text

Glycans, whose structures are not templated, are one of the four families of structurally related macromolecules that comprise living organisms, along with nucleic acids, proteins, and lipids. However, unlike DNA, RNA, and proteins, which possess predominately linear structure comprising a limited number of subunits with defined stereochemistry, glycans may exhibit incredibly complicated branched structures with a large number of subunits having both structural and stereochemical diversity. We have developed a toolbox of biomimetic reagents that use a combination of acid-base and free radical chemistry which are enabling for structural glycobiology. Noting the importance of proton transfer in the active site of many enzymes known to cleave glycosidic bonds, we developed a sequestered proton reagent for acid-catalyzed glycan sequencing (PRAGS) that derivatizes the reducing terminus of glycans with a pyridine moiety. Gas-phase collisional activation of PRAGS-derivatized glycans predominately generates C1–O glycosidic bond cleavages, retaining the charge on the reducing terminus. The resulting PRAGS-directed deconstruction of the glycan can be analyzed to extract glycan composition and sequence. Glycans are also highly susceptible to dissociation by free radicals, mainly reactive oxygen species, which inspired our development of a free radical activated glycan sequencing (FRAGS) reagent, which combines a free radical precursor with a pyridine moiety that can be coupled to the reducing. Collisional activation of FRAGS-derivatized glycans generates a free radical that reacts to yield both cross-ring and glycosidic bond cleavages, with charge retention at the reducing terminus. The approach developed here offers unique advantages compared to earlier studies employing mass spectrometry for glycan structure analysis. Related reagent chemistry is being developed to analyze glycopeptides, simultaneously providing glycan structure and peptide sequence information.

Keywords: Bioanalytical, Characterization, Electrospray, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Tandem mass spectrometry is now contributing in a significant way to structural biology studies. Ion mobility, which separates ions by size, shape, and charge, is a useful addition to these studies because it allows collisional cross sections to be measured for both precursor ions and product ions. In the work to be presented, surface induced dissociation (SID) is used to cause fragmentation of protein-protein, protein-RNA, and protein-DNA complexes. Ion mobility CCS measurements aid in interpretation of the dissociation results. Results will be presented for several systems under investigation, including protein-protein systems, RNase P, and nucleosomes. The influence of ion source conditions and charge on dissociation will be discussed and the SID results will be contrasted with CID (collision induced dissociation using gaseous target) results, illustrating that SID provides more information on the subcomplex structure.


Keywords: Bioanalytical, Mass Spectrometry, Nucleic Acids, Protein
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
This presentation will summarize the state-of-art in liquid chromatography - mass spectrometry (LC/MS) and related techniques with the main focus on recent developments in mass spectrometry and potential applications in the environmental analysis. Main configurations of modern mass spectrometers in LC/MS and MS along setups will be overviewed and critically compared in terms of their potential for the environmental analysis. Recent trends in LC/MS include the shift from low-resolution to (ultra)high-resolution tandem mass analyzers (mainly Fourier transform mass analyzers - Orbitrap and ion cyclotron resonance) providing also high mass accuracy (below 1 ppm), the shift from conventional high-performance liquid chromatography (HPLC) /MS to ultrahigh-performance liquid chromatography (UHPLC) /MS or other fast LC/MS techniques (core-shell particles, high-temperature LC and monolithic columns) requiring fast MS analyzers (typically time-of-flight based systems) and the use of multidimensional separations or multidimensional mass spectrometric analysis (e.g., mass spectrometry together with ion mobility spectrometry). The right LC/MS quantitation based on the use of suitable internal standards and dedicated scans (typically selected reaction monitoring, precursor ion and neutral loss scans) are very important for targeted environmental analysis of trace contaminants. Present LC/MS systems generate huge amount of analytical data, so dedicated softwares and data processing algorithms are essential for integrated omics approaches. The understanding of mass spectra interpretation is still important, so the basic rules for the interpretation of atmospheric pressure ionization mass spectra of small molecules will be presented and the typical fragmentation behavior for individual functional groups will be generalized.

Keywords: Electrospray, Environmental Analysis, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry

Application Code: Environmental

Methodology Code: Mass Spectrometry
Accurate Mass Analysis of Environmental Compounds with Both LC and GC/Q-TOF-MS

Application of TOF Mass Spectrometry and Sample Profiling Techniques to Water Analysis

Water scarcity due to climate change and growing urban population constrains more and more major cities to look for new water resources. Therefore, the possibility of reusing wastewater is increasingly considered worldwide. However, the major concern resides in removing known and unknown contaminants with potential impact on human health. Targeted analysis with triple quadrupole mass spectrometers indicate that advanced oxidation processes (AOPs) might play a major role in water treatment because of their ability to provide efficient and unspecific removal of organic contaminants through hydroxyl radicals. However, little is known on the fate of unknown contaminants and the formation of byproducts. The present study aims to address these points using time of flight mass spectrometry to generate the chemical profile of water samples before and after different AOP treatments. Wastewater effluent was collected at a local facility and circulated through a pilot allowing simulation of O3, UV, O3/UV, UV/H2O2 and O3/UV/H2O2 treatment. After solid phase extraction, compounds were analyzed by LC-QTOF with C18 column and electrospray ionization. The analysis of water extracts confirmed that a vast majority of compounds in the sample were unknowns. Indeed, more than a thousand compounds were detected in each extract. Sample profiling pointed out the compounds removed by each AOP but also the by-products formed consequently. Such differences could be easily visualized through a heat map indicating the relative abundance of each compound in each extract. In addition, sample profiling revealed similarities between samples allowing building clusters of compounds with similar behavior.

Keywords: Environmental, Environmental Analysis, Environmental/Water, Time of Flight MS
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
Accurate mass, using time-of-flight mass spectrometry (TOF) instruments with high resolution, is finally a widely accepted analytical technique and it has been the method of choice of many scientists in the environmental field for the identification of organic contaminants, such as pharmaceutical and pesticides in water samples. Lately, the increase in resolving power (>25,000) for LC-TOF-MS instrumentation has been one of the main achievements, thus opening up a wide range of possibilities for unequivocal identification and interference discrimination of environmental relevant compounds in complex samples. A comprehensive multi-residue screening method for the analysis of a large number of pharmaceuticals and their degradation products water samples is reported here. Furthermore, several tools, using accurate mass, have been successfully used to identify new and emerging contaminants in water samples. Some of these tools include the following: the use of molecular feature extraction based software, accurate mass databases, isotope filters, mass defect, use of accurate MS-MS fragmentation and mass profiling. These techniques were successful for finding non-targets and unknowns that had not been previously included in routine target methods. In this presentation, examples on how to maximize the use of these tools will be given for specific new findings in the environment. In addition, high resolution accurate mass using TOF was shown to discriminate between several isobaric/isomeric pairs of compounds (same nominal mass), frequently present in water samples.

Keywords: Environmental Analysis, Liquid Chromatography, Mass Spectrometry, Time of Flight MS
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
This paper describes the use of a GC-QTOFMS and a prototype soft ionization source to identify emerging contaminants [i.e., polycyclic aromatic sulfur heterocycles (PASHs), phosphate esters, pesticides] in selected certified standard reference materials and air samples. The soft ionization source incorporates a microplasma discharge, as a source of vacuum ultraviolet (VUV) photons, and can be tuned by choice of plasma gas to operate in the range of 104-150 nm, corresponding to photon energies between 8 and 12 eV. The ionization source utilizes a windowless design that allows operation with the full range of rare gas mixtures including those with emission lines below the ~105-115 nm cutoff of the VUV transmissible materials such as LiF and MgF2. In addition to the “soft” ionization mode, the ionization source described here can also be operated in a selective mode, where the photoionization energy is chosen to be less than the vertical energy of the solvent used for analysis or the components of the sample matrix. Several examples will be shown to illustrate the structure elucidation procedure using accurate mass and isotopic patterns provided by the TOF mass spectrometer as well as the N-rule, searching for candidate formulae in databases, and recognition of relevant substructures on the unknown compound.

Keywords: Environmental/Air, Fuels\Energy\Petrochemical, Gas Chromatography/Mass Spectrometry, Pesticide
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Accurate Mass Tools to Identify Hydroxy Radical Products of UV Oxidation of Pharmaceuticals

The combination of high resolution chromatography (UHPLC) and high resolution accurate mass (HRMS) is used to identify the hydroxy radical degradation products of five pharmaceuticals commonly found in wastewater. The pharmaceuticals included: carbamazepine, diphenhydramine, lamotrigine, trimethoprim, and venlafaxine. The five compounds were oxidized by a combination of hydrogen peroxide and UV at 254 nanometers in a matrix of wastewater. The loss of parent compound was followed using high resolution accurate mass by liquid chromatography/mass spectrometry using LC/QTOF-MS. The formation of degradation products was measured by the same technique using reverse phase chromatography as a guide to the substitution of hydroxyl radical for either hydrogen or methoxy groups present on the pharmaceutical. Basically, the parent pharmaceutical was used as a baseline for the longest retained degradation product and all degradates were found at retention times that were less by 2 to 4 minutes, which was caused by the substituted hydroxyl radical for a hydrogen or methoxy group. There was only one exception to this pattern, which was a metabolite of diphenhydramine that was one minute longer than the parent compound, in spite of the addition of a hydroxyl group to the molecule. This exception is examined as an example of how chemistry affects retention time and chemical structure. The hydroxyl substitution accounted for approximately 9% at the least for lamotrigine to 33-34% for carbamazepine and venlafaxine. The advantage of LC/QTOF-MS is that it is also possible to do MS-MS analysis for structural confirmation or, at the least, structural insights into the location of the hydroxyl substitution and estimates of the rates of formation and substitution that occur for these pharmaceuticals. A series of accurate mass tools are used and explained as a means to find and identify degradation products of UV oxidation.

Keywords: Environmental Analysis, Environmental/Water, Liquid Chromatography/Mass Spectroscopy
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
When the isotropic to nematic phase transition temperature of nematogens is approached from above, orientational relaxation slows dramatically. The slowing arises because the nematogens have pseudo-nematic order in the isotropic phase. Although the liquid is macroscopically orientationally isotropic, locally the molecules are not randomly oriented, but rather have a net projection on a local director. As the temperature is lowered, the correlation length of the pseudo-nematic domains grows and becomes infinite at the isotropic-nematic phase transition. An important question for systems with mesoscopic order is how much impact does the existence of ordering have on the individual molecules? Here ultrafast two dimension infrared (2D IR) vibrational echo experiments are used to investigate the temperature dependent structural dynamics experienced by molecules in pseudonematic domains by observing the time dependence of spectral diffusion. In addition, optical heterodyne detected optical Kerr effect (OHD-OKE) experiments observe the orientational dynamics of the bulk liquid. These experiments display the orientational relaxation slowing as the correlation length grows. The nematogen is 5CB (4-cyano-4'-pentylbiphenyl). Initially the CN stretch was studied with 2D IR, but the lifetime is short (4 ps) and severe heating occurs in the pure liquid. The heating problem was eliminated and a longer lifetime (8 ps) was obtained by making the first measurements of natural abundance carbon 13 CN. To extend the measurements to much longer times, two probe molecules, in which the CN in 5CB is replaced with SCN or SeCN, are used in low concentration as the vibrational probes. These molecules have long lifetimes (>100 ps). The OHD-OKE experiments demonstrate that the probes do not significantly modify the behavior of the isotropic phase. A comparison is made to a similar but non-nematogen liquid. The results show that the individual nematogen dynamics are not significantly influenced by the orientational correlation until very close to the isotropic-nematic phase transition where the correlation length becomes very long. Theoretical results using a mode coupling theory of the orientational dynamics of nematogens in the isotropic phase are discussed briefly.

Keywords: Infrared and Raman, Molecular Spectroscopy, Ultra Fast Spectroscopy, Vibrational Spectroscopy
Application Code: Materials Science
Methodology Code: Physical Measurements
Electronic structure and dynamics determine material properties and behavior. Two-dimensional (2D) optical spectroscopy has proven an incisive tool to probe fast spatiotemporal electronic dynamics in complex multichromophoric systems. However, acquiring these spectra requires long point-by-point acquisitions that preclude their use in unstable environmental conditions. Here, we discuss the development of single-shot coherent spectroscopy coupled to super-continuum generation to capture 2D ultrabroadband spectra in a single laser pulse. This tool may be used to investigate fundamental energy transfer dynamics in complex systems with multiple absorbers or as a general tool for characterization and identification of coupled molecular systems by optical spectroscopy.

Keywords: Absorption, Characterization, Laser
Application Code: General Interest
Methodology Code: Molecular Spectroscopy
Ultra-broad-bandwidth lasers are ideal sources for nonlinear spectroscopic applications given that they encompass all the necessary frequencies in one coherent beam. My group solved the challenge of ensuring that all the frequencies are in phase at the focal plane of the experiment, taking advantage of the phase dependence of nonlinear optics on phase. Using multiphoton intrapulse interference phase scan (MIIPS) we are able to routinely use sub-6fs pulses to solve challenging spectroscopic applications. This talk will focus on our progress towards explosives trace detection and confocal flame imaging. Both applications take advantage of pulse shaping for selective stimulated Raman excitation.

Keywords: Environmental, Identification, Laser, Raman
Application Code: Homeland Security/Forensics
Methodology Code: Molecular Spectroscopy
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**Primary Author**
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**Date**: Monday, March 03, 2014  -  Morning  
**Time**: 10:35 AM  
**Room**: S402b

**Abstract Text**
Two-dimensional infrared spectroscopy (2D IR) provides a platform for investigating structural properties and kinetics associated with DNAs. This presentation will present recent experimental and computational advances in the development of 2D IR for DNA, as well as applications to tautomerism and folding of DNA aptamers.

**Keywords**: FTIR, Nucleic Acids, Ultra Fast Spectroscopy, Vibrational Spectroscopy  
**Application Code**: Bioanalytical  
**Methodology Code**: Vibrational Spectroscopy
Protein conformational heterogeneity, motion, flexibility are thought to significantly impact protein function, but investigation of their contribution is limited by the availability of methods for characterizing rapidly fluctuating protein states with both high spatial and temporal resolution. Multidimensional infrared spectroscopy is emerging as a powerful technique that directly probes the structural dynamics of proteins on fast timescales. However, the spectral congestion inherent to protein spectra limits application of infrared spectroscopy to the study of protein systems. We incorporate into proteins vibrational probes with spectrally isolated frequencies and local-mode character that make possible rigorous analysis of protein environments and dynamics with infrared spectroscopy. In particular, both heme-bound carbon monoxide and nitriles selectively incorporated as unnatural amino acids were used as probes of cytochrome P450. The application of these methods to investigate the contribution of protein dynamics and conformational heterogeneity to the specificity of its catalytic activity will be discussed. In addition, studies of mutants of cytochrome P450 shed light on the specific protein residues that contribute to the protein’s dynamics.
The application of state-of-the-art analytical technologies has become immensely important for the detailed annotation of disease states. Especially, pertinent has been the “biomarker”, a measurable characteristic that can reflect the severity or presence of a disease state. Within the sphere of disease related biomarkers, we have developed two LC-MS/MS-based methods for biomarker quantification and identification in parasitic (Onchocerciasis) and bacterial (Pseudomonas aeruginosa) infections. Onchocerciasis, also known as "river blindness", is a neglected tropical disease infecting millions of people mainly in Africa and the Middle East but also in South and Central America. Currently, there is no point-of-care diagnostic that can distinguish the burden of infection. We have identified, synthesized and quantified a unique biomarker, N-acetyltyramine-O,β-glucuronide (NATOG), a neurotransmitter-derived secretion metabolite from O. volvulus by urine metabolome analysis. Importantly, the regulation of this neurotransmitter was linked to patient disease infection. The prominence of this biomarker, its application as a diagnostic and its relationship to O. volvulus monitoring will be discussed. P. aeruginosa is an opportunistic pathogen that colonizes the lungs of patients with Cystic Fibrosis (CF), which leads to progressive lung damage, respiratory failure and eventually death. This bacterium employs a specific set of chemical compounds known as quorum sensing (QS) autoinducers to synchronize gene expression, ward off microbial competitors and to ultimately establish an infection. The concentration dependence of these QS molecules with the progression of bacterial disease state suggested an opportunity for diagnostic development. Two molecules that P. aeruginosa utilizes in QS were detected and quantified in sputa of patients with CF obtained during different clinical states. The basis of autoinducers for the monitoring of disease states will be detailed.

Keywords: Biomedical, Liquid Chromatography/Mass Spectroscopy
Application Code: Biomedical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Molecular Analysis of Human Disease

Single Molecule Arrays for Early Disease Detection

Disease detection and diagnosis are constrained by the detection limits of conventional immunoassays, such as ELISAs. Our premise is that one should be able to detect disease at a much earlier stage if one could detect a biomarker signature earlier in the disease progression cycle. By using ultra-sensitive single molecule protein assays, we are measuring protein biomarkers at 100-1000 times lower limits of detection than ELISAs. These assays are directed at detecting both breast cancer and infectious disease much sooner with an ultimate goal of earlier intervention leading to better clinical outcomes.

Keywords: Bioanalytical, Biological Samples, Immunoassay, Proteomics
Application Code: Clinical/Toxicology
Methodology Code: Other (Specify)
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 may provide complementary information to mass spectrometric (MS) methods that are commonly used in clinical glycomics. N-glycans are enzymatically cleaved from glycoproteins in serum and derivatized with 8-aminopyrene-1,3,6-trisulfonic acid to impart needed charge for electrophoresis and a fluorescent label for detection. The microfluidic devices generate electrophoretic separations with analysis times under 100 s, efficiencies up to 700,000 plates, and migration time reproducibilities better than 0.1% RSD after peak alignment. Statistical analyses reveal differences among the N-glycan profiles of the various sample groups. To better understand alterations in the glycomic profile associated with disease progression, we are also comparing results for the same samples analyzed by microchip electrophoresis and MALDI-MS.
While metastatic disease causes ~90% of all cancer related deaths, many diagnostic tests have difficulty in determining whether a patient is metastatic or pre-metastatic, the determination of which can have a profound impact on the treatment modality. For example, pancreatic ductal adenocarcinoma (PDAC) is an aggressive disease that has a poor overall survival rate and different treatment regimens are required for those with metastatic (chemotherapy) versus non-metastatic (surgery and chemotherapy) disease. To assist the oncologist in determining the course of treatment for PDAC patients, an integrated microfluidic system was designed, fabricated and evaluated. The system could select from whole blood circulating tumor cells (CTCs) comprising different phenotypes, enumerate and immuno-fluorescently identify the CTCs. The integrated system consisted of a CTC selection unit, an electrical detector for CTC counting, an imaging unit for phenotyping single cells and a molecular analysis unit for detecting mutations or expression profiling the CTCs. The CTC selection unit contained a series of 190 nL fluidic channels the walls of which were decorated with antibodies used to recognize and select various types of CTCs from a single blood sample. The cell selection unit could process 7.5 mL of whole blood in ~20 min with a recovery of 97% and purity >80%. The CTCs were subsequently released from the surface-immobilized antibodies and initially counted by measuring impedance signatures of single cells that traversed through a pair of electrodes. The CTCs were then stained and phenotyped with the appropriate cells subjected to molecular analysis. The use of the system for determining the molecular characteristics of CTC sub-populations were evaluated for PDAC patients to determine which patients should go to surgery and also, monitor the patient’s response to therapy.
Huntington’s disease (HD) is a fatal, genetic, neurodegenerative movement disorder for which there is no cure or effective treatment. HD is caused by a CAG repeat expansion on the gene that encodes the huntingtin protein, resulting in the expression of an expanded N-terminal polyglutamine segment. The striatum, a brain region rich in dopaminergic innervation, is among the first to degenerate in HD. Our research group has investigated alterations in striatal dopamine release in multiple HD model rodents. Using fast-scan cyclic voltammetry, we have found that dopamine release, and, in some cases uptake, is impaired. Additionally, these release impairments correlate with alterations in locomotor activity and gait. Moreover, release impairments are a result of either incomplete vesicle loading or decreased number of vesicles. More recently, we have found that release impairments are region specific within the striatum, and that serotonin release is also impaired. The implications of these findings will be discussed.

Keywords: Bioanalytical, Electrochemistry, Microelectrode, Neurochemistry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
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**Abstract Text**

For the past 30 years, the general appearance of gas chromatography (GC) instrumentation has remained virtually unchanged. During this period, there have been significant improvements in column technology, sample introduction devices, detectors and data handling software; however, the large, bulky convection oven has persisted as the age-revealing characteristic of the gas chromatograph. In recent years, new GC instrumentation has been slowly evolving, driven primarily from interest in (1) a smaller laboratory bench footprint, (2) faster analysis, (3) higher resolving power (i.e., comprehensive GCxGC), and (4) field-portable instrumentation. This evolution has been facilitated to some degree by new developments in resistive heating and micro-fabrication technologies. Tools are now available and opportunities are now emerging to develop truly “smart” GC instruments and methods. Modern reincarnations of thermal gradient GC, a unique GC technique that was originally reported in the 1960s, provide simple approaches to achieving smart GC.

**Keywords:** Capillary GC, Gas Chromatography, GC, Instrumentation

**Application Code:** Other (Specify)

**Methodology Code:** Gas Chromatography
Direct resistive heating methods have long been reported as alternatives to air baths and heater blocks but fragile electrical connections, losses of efficiency and inferior reproducibility have kept resistive heating of columns from being popular for most applications. Valco Instruments has developed a temperature controller with sophisticated control algorithms for direct resistive heating using a nickel wire or exterior nickel plating on the column as both heater and sensor. Columns are microchip encoded to enable control and programming from 1/3-30 meters. Using this technique has reduced the column separation efficiency loss to only 2-4%. Power consumption is significantly reduced from air bath heating. For example, only 15 watts are required to heat a 5-meter column to 300°C at 200°C/min. Since the heater and sensor are the same nickel element and a sophisticated algorithm is used, overshoot is avoided during fast programming, consequently the lifetime of the column may be increased compared with other resistive heating techniques. For example, in a simulated distillation application, we heated a column from 40°C to 360°C in 2min separating n-C5 to n-C44 hydrocarbons, and cooled back to 40°C in 1.5min.

Also, the temperature controller may be used for other GC components, such as fittings, transfer lines, injectors and detectors. The typical mass of these components has prevented their ability to be rapidly programmed and limited the adoption of resistive column heating. We have developed reduced mass components and demonstrated superior temperature control and rapid heating with or without separate sensors. For example, we have developed a low mass cold on column injector and interface coupled to a resistively heated column. The injector is heated resistively at up to 1000°C/min or programmed to track the analytical column. It also cools rapidly from 400°C to 40°C in less than one minute.

Keywords: Capillary GC, Gas Chromatography, High Temperature, High Throughput Chemical Analysis
Application Code: High-Throughput Chemical Analysis
Methodology Code: Gas Chromatography
Generation of a high peak capacity is critical for the analysis of complex samples using one-dimensional and two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC – TOFMS and GC x GC – TOFMS). Furthermore, producing a high peak capacity in a short separation time is necessary to provide high throughput, to address emerging challenges in chemical analysis. In this presentation, recent advances in instrumentation development will be reported for GC – TOFMS and GC x GC – TOFMS, with separations in the ~ 6 to 7 minute time frame providing a total peak capacity of 600 peaks (at unit resolution), corresponding to a peak capacity production of 100 peaks/min in GC – TOFMS, and a total peak capacity of ~ 6000 peaks in GC x GC – TOFMS, corresponding to a peak capacity production of approaching 1000 peaks/min. Novel computational methods are also being explored to dramatically improve the effective peak capacity of GC – TOFMS and GC x GC – TOFMS. These recently developed computational methods will be reported.

Keywords: Capillary GC, Data Analysis, High Throughput Chemical Analysis, Time of Flight MS
Application Code: High-Throughput Chemical Analysis
Methodology Code: Gas Chromatography/Mass Spectrometry
The development and preliminary characterization of a microfabricated comprehensive two-dimensional gas chromatographic system (µGC×µGC) comprising Si-micromachined components are described. The heart of the system is a microfabricated thermal modulator (µTM). The 2-stage µTM chip (13×6×0.5 mm) consists of two interconnected spiral etched-Si microchannels (4.2 and 2.8 cm long; 250×140 µm c.s.), an anodically bonded Pyrex cap, and a 0.3-µm-thick crosslinked wall coating of PDMS. Integrated heaters provide rapid, sequential heating of each µTM stage at rates as high as 2400 °C/s, while a proximate, underlying thermoelectric cooler provides continual cooling. The average power is only 10 W for heating and 21 W for cooling without using consumable materials. Initial testing with commercial 1st- and 2nd-dimension capillary columns (PDMS and PEG phases, respectively) explored the effects of various µTM design and operating parameters on performance and yielded excellent results. Multi-physics modeling is being used to guide efforts to design a 2nd-generation µTM. Subsequently, we used a series of two etched-Si µcolumns (3×3 cm footprint; 250×140 µm c.s.; 3-m length per chip) with wall-coated PDMS stationary phases as the 1st dimension, and a single etched-Si µcolumn (1.2×1.2 cm footprint; 150×50 µm c.s.; 0.5-m length) with a wall-coated trigonal tricationic room-temperature ionic liquid (RTIL) stationary phase as the 2nd dimension. Isothermal and temperature-ramped operation was explored. Results, to date, include a structured 7-component µGC×µGC chromatogram and a 10-min, 17-component separation. Strong retention of polar analytes by the RTIL in the 2nd dimension causes broadening of peaks and loss of resolution, which is being mitigated by adjustment of the phase thickness. The advantages and remaining challenges to optimizing the µTM, the µcolumns, and the system integration for low-resource µGC×µGC separations of more complex mixtures will be discussed.

Keywords: Capillary GC, Environmental Analysis, GC Columns, Lab-on-a-Chip/Microfluidics
Application Code: Environmental
Methodology Code: Microfluidics/Lab-on-a-Chip
Isothermal conditions are well known to produce optimal separations of analytes with similar retention. Temperature programming methods are used to reduce analysis time when the sample contains analytes with widely different retentions. Although strictly sub-optimal as measured by resolution, temperature programming has a variety of properties that make it the method of choice for most GC applications. Similarly, thermal gradient gas chromatography (TGGC) possesses a variety of properties of interest to the analyst that favor this method for certain applications. Some of these properties include virtual column length, correction of imperfect separation, restriction of peak broadening over time, straightforward application to 2-D separations, increase in peak capacity for short columns, low back pressure, on-column concentration of analytes, and on-demand analyte elution. Various properties of moving thermal gradient gas chromatography (TGGC) separations were examined using the Markov random walk model of Giddings. Subsequent TGGC separations were found to support the fundamental theory and illustrate the unique advantages of this separation technique.
Expanding the Capabilities of Mass Cytometry

The Mass Cytometer is a specific, designed-for-purpose implementation of an analytical atomic mass spectrometer. The principal point of commonality is the Inductively Coupled Plasma that provides atomization and ionization. This presentation will focus on the unique adaptations that address the need for single cell distinction, with the sensitivity and dynamic range that meet the needs of biologic informatics. The overall efficiency of a Mass Cytometer system in quantifying antigens in real time single cell assays depends on several factors. First, processes of aerosol generation, vaporization, atomization and ionization of single cells define how quantitatively the single-cell induced ion cloud sampled through the plasma-vacuum interface represents the composition of each single cell. Second, ion transport through the interface, the ion optical path and the time-of-flight analyzer define not only the absolute sensitivity (effectively defining the minimum number of copies of antigen per cell that can be detected given a specific cell-labeling efficiency), but also how readily bright and dim labels can be distinguished while retaining the specificity and high multiparameter capability of the assay. Third, ion signal handling on the nano-second scale, which includes ion detection, signal pre-amplification, digitization and processing, defines the dynamic range of a single cell assay. Integration of the ion signals over the mass-time windows, combined with appropriate real-time Gaussian curve fitting of the sequential spectra corresponding to microsecond scale transients, provides additional gating information to resolve doublets and debris from true single cell events. Using leukemia cell lines, we will demonstrate how the theoretical fundamentals of these sequential processes can be translated into hardware and software improvement of data fidelity.

Abstract Text

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Keywords: Bioanalytical, ICP-MS, Instrumentation
Application Code: Bioanalytical
Methodology Code: Other (Specify)
In the past 10 years, both clinical and basic biology research were revolutionized by “omics” platforms that capture a nearly complete body of biomolecular information, such as the relative levels of every transcribed mRNA (the transcriptome), or the sequence of every gene (the genome). Typically, such “omics” platforms require the molecular material from millions of cells, blended together into a homogenous cell lysate. Biological systems, however, are complex, heterogeneous, and dynamic collections of cells with different roles, agendas, and sometimes – as in the case of cancer – different mutations that endanger the host. Homogenization discards the information that exists at the granularity of single cells. A major remaining frontier in biological research is to capture a large amount of information at the single-cell level, and to computationally integrate that information with other analysis platforms, to better understand the biological system as a whole.

New analysis platforms, such as automated quantitative microscopy, microfluidics-based approaches, and CyTOF mass cytometry have begun to provide a rich layer of single-cell information. While the data from these platforms is far from a comprehensive snapshot of cellular heterogeneity, it nevertheless represents a dramatic shift in the level of understanding we can gain about normal and disease systems by examining their single-cell constituents. Major analytical and computational challenges – opportunities for innovation – will arise as this new class of data grows in depth and scope. In this presentation, three emerging high-parameter sample analysis approaches will be briefly reviewed. Real-world examples from our research into normal immunology and cancer biology using 32-parameter CyTOF mass cytometry, and early efforts to integrate this data with other “omics” platforms, will be discussed.
New advances in high dimensional mass cytometry enable systems level analysis of protein expression, signaling network activity, and cellular functional outcomes in individual cells from primary tissues. A 'routine' experiment might now simultaneously measure 30 or more features: 12 surface markers of cell identity, 14 phosphorylation states of signaling molecules, and 4 functional outcomes (e.g. protein expression, cell cycle stage, necrosis, apoptosis). Data from this type of analysis can then be used to create a network map of signaling in each of the millions of cells collected.

We are using this single cell systems biology approach to dissect signaling network control of healthy development in human cells and to model how disease disrupts signaling network architecture. The flood of high dimensional single cell data generated by this approach has also created a need for new computational tools to model signaling networks and to classify and track cell populations based on complex phenotypes. In particular, tools are needed that 1) automatically identify novel populations of cells in heterogeneous samples, 2) visualize the markers measured across populations of cells, individuals patients, time, drug doses, and other variables, and 3) provide a platform that connects investigators with diverse skill sets to datasets and computational resources.

Advances in single cell systems biology could have a profound long term impact on scientific research and human health. By understanding the biological systems controlling development and cell-cell interactions, we can learn to program cells to become therapeutic agents or precisely target abnormal signaling to specifically kill diseased cells.

Keywords: Biological Samples, Biomedical, Immunoassay, Proteomics
Application Code: Biomedical
Methodology Code: Other (Specify)
The focus in cancer research on the malignant cancer cells has recently been extended to include the tumor microenvironment (TME). It contains different cell types such as infiltrating immune cells and stromal fibroblasts, which can support tumor progression and drug resistance. Consequently, an understanding of the interactions between tumor and TME will contribute to the identification of novel therapeutic targets.

Mass cytometry enables high-dimensional analysis of cell type and state at single-cell resolution, which will be needed to study the interactions between tumor and TME. However so far, mass cytometry has been exclusively used to analyze cells in suspension. In order to comprehensively gain information on cell location and cell-to-cell interactions in a complex disease microenvironment, we have coupled immunohistochemical methods with high resolution laser ablation and mass cytometry. This novel imaging approach enables the simultaneous visualization of currently 32 proteins and protein modifications at subcellular resolution with the potential to map up to 100 markers on a single tissue section.

Imaging mass cytometry presented herein provides comprehensive analysis of adherent cells and formalin-fixed paraffin-embedded (FFPE) samples. As such it has the potential to yield novel insights from existing large collections of FFPE tumor samples and associated clinical information. The visualization of single cell marker expressions in breast cancer tissue sections revealed a rich complexity of cell phenotypes and cell-to-cell interactions. Using an in-depth view on cancer heterogeneity, this next generation tissue-based imaging method may be valuable for the classification of cancer and support the transition of medicine towards individualized molecularly targeted therapies.

Keywords: Biological Samples, Biomedical, Elemental Mass Spec, Imaging
Application Code: Biomedical
Methodology Code: Mass Spectrometry
Cholesterol efflux from the cell plasma membrane is known to be increased in cell-line and mouse models of cystic fibrosis. A noninvasive means of characterizing plasma membrane cholesterol efflux at affected tissues is needed to extend the trends found in models of cystic fibrosis to humans suffering from the disease. Microelectrode induced cholesterol efflux from the plasma membrane surface of tissue is proposed as a strategy to demonstrate increased cholesterol efflux for cystic fibrosis in human subjects. Data demonstrating detection of cholesterol efflux from the human buccel mucosa is reported as proof-of-concept for the in vivo diagnostic assay. Cholesterol oxidase modified Pt microelectrodes, positioned in contact with the mucosa, show increased signal relative to control electrodes indicating observation of cholesterol efflux from mucosa surface cells. Electrochemical detection of cholesterol involves accumulation of enzymatically produced hydrogen peroxide at the electrode surface and periodic oxidative depletion of peroxide in a series of potential step experiments. A nano-electrode array strategy is proposed as a means of increasing signal-to-background of these measurements. This work was supported by NIH 1R01EB009481.

Keywords: Biomedical, Biosensors, Detection, Electrodes
Application Code: Biomedical
Methodology Code: Sensors
In the summer of 2008 the Wet Chemistry Lab (WCL) onboard NASA’s Phoenix Mars Lander performed the first electrochemical analyses of the soluble species and solution parameters for three 1-gram samples of martian soil added to 25 mL of water. The WCL consisted of an array of electroanalytical sensors that included ion selective electrodes (ISE) to measure the concentrations of Mg\(^{2+}\), Ca\(^{2+}\), Na\(^{+}\), K\(^{+}\), Cl\(^{-}\), Br\(^{-}\), I\(^{-}\), NO\(_3^-\), SO\(_4^{2-}\), and H\(^{+}\). It also contained electrodes to determine solution conductivity, redox potential, pH, and to run cyclic voltammetry (CV) and chronopotentiometry (CP). The analyses revealed an alkaline soil containing a variety of soluble minerals, and unexpectedly almost 1% perchlorate (ClO\(_4^-\)), a discovery that has altered the way we view the chemistry of Mars, its potential to support past or present indigenous life, and future human exploration and habitability.

The discovery of ClO\(_4^-\) also lead to analyses of soils in the Antarctic Dry Valleys (considered Mars analog sites) that provided the first unambiguous evidence of the ubiquitous natural formation of ClO\(_4^-\) on Earth, with accumulation in arid environments and global atmospheric production.

The ClO\(_4^-\) ISE in conjunction with the effects of the ClO\(_4^-\) on the Ca\(^{2+}\) ISE allowed the determination of the ClO\(_4^-\) parent salt as being dominated by Ca(ClO\(_4\))\(_2\), a finding which implies a more severely arid Mars than assumed. The WCL vividly demonstrates the ability and power of a simple array of electroanalytical sensors to provide a unique picture of aqueous geochemistry and potential biohabitability in a variety of extreme or remote terrestrial and extraterrestrial environments.
Although bioelectrocatalytic biosensors have been developed for a variety of biomedical applications, they also have a wealth of applications in analysis of waste streams, including: waste water and storm water analysis, analysis of biofuel production waste streams, and analysis of beverage production waste streams. This paper will discuss the sampling issues with different waste streams and compare the different types of bioelectrocatalysis for the different waste stream applications. These types of bioelectrocatalysis will include enzymatic, thylakoid, and mitochondrial bioelectrocatalysis in both direct and mediated electron transfer modes.
Trace metal pollution is an important environmental and public health issue. The adverse effects of toxic metals are well-documented and there is great interest in reducing their impact. To efficiently implement mitigation systems, more information is needed about dynamic metal behavior in natural water systems such as stormwater. This necessitates an in-situ analytical device that is portable, low-cost, low-energy and submersible while providing a continuous measurement output. While electrochemistry has shown promise for this goal in the past, it has been limited by its temporal resolution and concerns about Hg toxicity. In this work, a novel voltammetric technique that can perform Hg free, sub-second, real-time metal analysis at carbon fiber microelectrodes is described. With an array of analytical tools including XPS spectroscopy and AFM imaging, we determine the surface catalytic mechanisms of fast metal voltammetry (FMV) for Cu. We show that Cu FMV is adsorption, not diffusion controlled. We establish the nature of the Cu complexes adsorbed on the electrode surface and the redox couple that gives rise to our electrochemical signal. Additionally, we construct adsorption isotherms for Cu on carbon fiber microelectrodes. Finally, we describe the application of FMV to real-time analysis of Cu and Pb in model and authentic stormwater samples.
Over the past few years, our group has developed the ability to use multiple electrochemical sensors to record cellular metabolic fluxes in a micro and nanofluidic environments. We have named this technique multianalyte microphysiometry based on the original commercial method of acidification microphysiometry. The instrumentation developed includes the creation of new Labview controlled multipotentiostats that can measure three analytes in eight different microfluidic chambers simultaneously in approximately 3 microliters or less of total solution volume. New developments include the ability to combine four or more electrochemical sensors, either-potentiometric or amperometric, into microliter sized cell chambers. These multianalyte microphysiometers are being used to develop toxicity screening based on the organs-on-a-chip approach for an array of organ and tissue systems including neurons/glia, lymph nodes, and an artificial liver.

We are also pursuing rapid prototyping of these microfluidic devices in a lab-on-a-chip based format with multiple chambers on the same PDMS/glass chip with commercial screen printed platinum electrodes as well as our custom electrode arrays. These new prototypes allow for a wide range of electrochemical sensor arrays with enzymatic or ion-selective films on each array electrode while also allowing us to measure the cellular bioenergetics, i.e. the metabolic rates, of the tissues in the microfluidic chamber. This dimensional control enables the modeling of the extracellular electrochemical readouts in terms of electrochemical time of flight, the time from cellular release or uptake until the time of recording at the sensors.

Keywords: Biosensors, Clinical/Toxicology, Electrochemistry, Lab-on-a-Chip/Microfluidics
Application Code: Clinical/Toxicology
Methodology Code: Electrochemistry
The phenomenon of surface-enhanced infrared absorption (SEIRA) was discovered by accident by Hartstein et al. [1] who deposited a thin layer of p-nitrobenzoic acid (PNBA) on an internal reflection element on which silver had been vapor deposited and measured bands in the 3000-2800 cm⁻¹ region that were clearly not caused by PNBA. Like many subsequent investigators, these authors ascribed the enhanced absorption to a plasmonic mechanism analogous to surface-enhanced Raman scattering (SERS). A decade later, three groups showed that similar enhancements could be obtained from “island films” of metals other than Ag or Au. For all metals, it has been found that once the percolation limit has been reached, i.e., when the metal islands start to touch, the absorption bands in the spectrum start to take on some dispersive character. This behavior cannot be explained by a plasmonic mechanism and appear to be better explained by the Bruggeman representation of effective medium theory. Indeed Su et al. [2] have shown that the spectrum of CO adsorbed on platinum is exactly modeled with this formalism. In this talk, I will summarize the various mechanisms that have been proposed for SEIRA and attempt to conclude which gives the best explanation for the measured spectra.


Keywords: FTIR, Infrared and Raman, Nanotechnology, UV-VIS Absorbance/Luminescence
Application Code: Nanotechnology
Methodology Code: Vibrational Spectroscopy
Infrared spectroscopy is a benchmark analytical tool for identifying molecular vibrations at their mid-infrared frequencies. Surface-enhanced infrared absorption (SEIRA) increases the sensitivity of this technique by several orders of magnitude, such that ultrasmall quantities of molecules can be detected. In this work, we have used individual gold nanoantennas with commercial infrared spectroscopy to experimentally demonstrate attomole detection of octadecanethiol and zeptomole detection of hemoglobin. The antennas consist of four rods oriented perpendicularly with a common junction. This design allows us to use all polarizations of light for producing a focused region of enhancement that can be tuned to any molecular frequency by simply changing the length of the rods. The antennas were fabricated on ZnSe optical windows using electron beam lithography, which allows precise control of the structure geometry for transmission-based measurements in a Fourier-Transform Infrared (FTIR) microscope. An additional step of lithography was performed to deposit SiO$_2$ in selected regions to probe areas of maximum field enhancement. By comparing experimental data with simulations using the Finite-Difference Time Domain (FDTD) method, we found that 45% of the signal came from the junction, which represented 1 amol of octadecanethiol and 10 zmol of hemoglobin. The maximum SEIRA enhancement factor $|E/E_o|^2$ ranged between $10^3$-$10^4$. To increase sensitivity, we have recently developed a new antenna design with enhancement factors of more than $10^6$. The results from this study open up strong possibilities for using infrared spectroscopy to analyze single molecules. This work was supported by the Robert A. Welch Foundation, the AFOSR, the NSF, the DTRA, the NSSEFF, and the ARO.

Keywords: FTIR, Microspectroscopy, Nanotechnology, Vibrational Spectroscopy
Application Code: Nanotechnology
Methodology Code: Vibrational Spectroscopy
Membrane proteins are the target of more than 50% of all drugs and are encoded by about 30% of the human genome. Electrophysiological techniques, like patch-clamp, unravelled many functional aspects of membrane proteins but suffer from structural sensitivity. We have developed Surface Enhanced Infrared Difference Absorption Spectroscopy (SEIDAS) to probe potential-induced structural changes of a protein on the level of a monolayer [sup]1[/sup]. A novel concept is introduced to incorporate membrane proteins into solid supported lipid bilayers in an orientated manner via the affinity of the His-tag to the Ni-NTA terminated gold surface [sup]2,3[/sup]. Functionality was probed by binding assays and electrochemistry.

General applicability of this novel methodological approach is shown by tethering plant photosystems I and II to the gold surface [sup]4[/sup]. In conjunction with hydrogenase, the stage is set towards a biomimetic system for H2-production [sup]5[/sup].

Recently, we succeeded to record IR difference spectra of a monolayer of sensory rhodopsin II under voltage-clamp conditions [sup]6,7[/sup]. This approach opens an avenue towards mechanistic studies of voltage-gated ion channels with unprecedented structural and temporal sensitivity [sup]8[/sup].

References:

Keywords: Bioanalytical, Biospectroscopy, Spectroelectrochemistry, Vibrational Spectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Surface-Enhanced Infrared Absorption: Mechanism and Applications

Application of SEIRAS to Mechanistic Studies of Electrocatalytic Reactions Related to Fuel Cells

SEIRA is similar to SERS in nature, but a significant difference exists between them in the metals available. SERS can be observed essentially only on coinage metals, while SEIRA can be observed not only on coinage metals but also on many other metals including Pt group metals. Taking this advantage, SEIRAS in the ATR mode has been applied very successfully to mechanistic studies of electrocatalytic reactions on Pt and Pt-group metals. The high surface selectivity and sensitivity allows us in-situ, real-time monitoring of reaction processes occurring at the catalyst surface under potential dynamic conditions [1]. The mechanisms of two simple (appears to be simple) electrocatalytic reactions, hydrogen evolution reaction [2] (the cathodic reaction in water hydrolysis) and formic acid oxidation [3] both on Pt electrodes revealed by SEIRAS, will be discussed together with some recent developments in experimental procedures.

References

Keywords: Electrochemistry, Electrode Surfaces, FTIR, Spectroelectrochemistry
Application Code: General Interest
Methodology Code: Molecular Spectroscopy
Surface-enhanced infrared absorption (SEIRA) spectroscopy has attracted considerable attention owing to its potential as a sensitive analysis tool in widespread applications. To successfully apply SEIRA effect for chemical analysis, both the production of suitable nanostructures and the immobilization of these nanostructures on IR sensing elements are critical. To effectively produce nanostructures directly on IR sensing elements, methods based on chemical and photo reductions have been proposed and examined their performances in production of suitable SEIRA nanostructures. Silver and gold nanostructures are mainly focused because they are either larger in SEIRA effect or better in biocompatibility as compared to other metals. The formation of the sensing phase by these nanostructures is also carefully designed to have enough porosity within the nanostructures to ensure fast detection. With multi-dimensional structure of the sensing phases, the loading capacity for sampling can be largely increased and hence, the sensitivity in detection. Selectivity in IR detection is usually based on unique IR absorption features of the targeted compounds. In SEIRA measurements, functionalization of the nanostructures can be used as a second mechanism to improve the selectivity in detection as the modified chemicals can selectively attract the targeted compound. Meanwhile, a band-shifting technique can be applied in sensing phase composed with functionalized nanostructures, which significantly improves the sensitivity and selectivity in detections.

Keywords: FTIR, Molecular Spectroscopy, Nanotechnology, Sensors
Application Code: Nanotechnology
Methodology Code: Vibrational Spectroscopy
This talk will outline recent progress in the development of ion sensors our laboratory. Spiropyran based chromophores are activated by light, which results in a very large increase in basicity. This can be used to develop light activatable optical sensors that brings the field closer to that of dynamic sensors. This particular example results in a shift of the dynamic cooperation or competition between protons and the analyte ion for the available binding sites, giving rise to ion extraction that can be triggered by light.

We also show that light activation can trigger ion fluxes across membranes, resulting in a current response that is attractive for the development of light harvesting systems. Such light triggered current responses can serve for the development of photodynamic sensing and extraction systems and may be interesting in energy conversion applications.
The exquisite selectivity of receptors of biological origin, such as antibodies, nucleic acids and enzymes, enabled the development of biosensors and high throughput biochip technology. Despite of their many advantages bioreceptors have also limitations in terms of chemical and temperature stability, as well as functionality restricted to their natural milieu. Therefore, synthetic receptors with enhanced robustness and tailored physical-chemical properties would address unmet needs. Here we are going to present new synthetic polymeric materials and receptors for selective recognition of proteins. Specifically, molecularly imprinted polymers prepared by using a combination of lithography and surface molecular imprinting with very good selectivities for the template proteins. Additionally, we will present new developments in spiegelmer selection (a form of aptamers that are mirror images of natural oligonucleotides) that enables their use for selective biomarker recognition in serum.

References

Keywords: Biosensors, Electrochemistry, Immunoassay, Materials Science
Application Code: Bioanalytical
Methodology Code: Electrochemistry
A method has been developed and validated for the analysis of nitrate in fresh and saline waters using calibration-free coulometric analysis employing a tubular membrane ion-selective electrode (ISE). In this paper, we will show that the detection of sub-micromolar levels of nitrate in natural waters is possible, and the new ISE method is sufficiently selective for the analysis of nitrate in fresh waters. However, in saline waters, the chloride interference is insurmountable, and must be eliminated by sample treatment using a specially designed sample electrolyzer to reduce chloride to analytically acceptable levels. Ultimately, we have developed a coupled electrolysis system/tubular membrane ISE for the analysis of nitrate in seawater, and this analytical system has been validated in artificial and certified seawater, as well as Port Phillip Bay seawater from Victoria. Last, we have undertaken a shipboard field trial of the integrated analytical system for the analysis of nitrate in the Derwent River Estuary in Tasmania, and these results will be presented.
We have been investigating direct interaction between biomolecules and electrons in a solid-state substrate. We have developed several types of bio-transistors (Bio-FETs) which are based on the direct transduction of charge density change of biomolecules into electrical signal by the field effect. One of the unique features of our method is to utilize a single-base extension reaction on the gate for DNA sequencing. Single-base mismatch of the target DNA as well as DNA sequencing could be successfully demonstrated with the use of the genetic FETs.

We also propose an oocyte-based field effect transistor (oocyte-based FET) for drug transport analysis, in which target transporters are expressed at the cell membrane of the oocyte. Non-invasive monitoring of the uptake kinetics of substrates mediated by membrane-bound transporters can be realized with oocyte-based FET. Discrimination of transporting ability among genotypes of the transporters could be realized using the oocyte-based FET.

A label free, potentiometric method to detect cell surface sialic acid (SA) using phenylboronic acid (PBA) compound integrated into the form of self-assembled monolayer (SAM) has been developed using a field effect transistor (FET) extended gold gate electrode. Due to predominant binding between undisassociated PBA and SA at pH 7.4, we found that carboxyl anions of SA were exclusively detectable among other glycan chain constituent monosaccharides, as the change in threshold voltage (VT) of the PBA-modified FET. The technique was applied to analyses of altered SA expressions on rabbit erythrocyte as a model for diabetes. The comparative analyses revealed that the disease could be feasibly diagnosed simply by placing the cell suspensions onto the device without any labeling and enzymatic procedures. The platform based on the biotransistors is suitable for a simple and inexpensive system for clinical research and point of care testing.

**Keywords:** Biosensors, Chemically Modified Electrodes, Electrode Surfaces, Ion Selective Electrodes

**Application Code:** Biomedical

**Methodology Code:** Sensors
For feedback controlled monitoring of the popular anesthetic drug propofol we used an organic membrane-coated voltammetric sensor. The membrane coating prevents electrode fouling, and provides outstanding detection limit and selectivity for the voltametric working electrode that is adequate for continuous monitoring of propofol in whole blood in the presence of physiologically relevant interferences.

The detection limit of the voltametric sensor is controlled by diffusion coefficient (D) and the concentration of the analyte in the membrane ([c[sub]M[/sub]]) where c[sub]M[/sub] is a function of membrane/solution partition coefficient (P). The selectivity of the membrane-coated sensor, on the other hand, depends on the partition coefficients of the analyte and interfering compounds.

To assess the attainable detection limit and selectivity of the membrane coated sensor the diffusion and the partition coefficients have to be known. For the determination of the diffusion coefficients in the membrane it has been loaded with the analyte and the steady state current of a membrane coated planar electrochemical cell with a carbon fiber ultra microelectrode has been recorded in air (D=i/nF[c[sub]M[/sub]])). By placing the membrane-coated planar electrochemical cell into a solution the concentration of the analyte in the membrane will be determined by its partition coefficients. From cyclic voltammetric experiments with the membrane-coated sensor in an analyte solutions one can calculate the partition coefficient of the analyte.

In our contribution we discuss the unique advantages of the voltammetric method and report the partition coefficients of a variety of compounds (e.g., propofol, ascorbic acid, p-acetamido phenol) between plasticized PVC membranes and aqueous electrolytes as well as the diffusion coefficients of these compounds in the membranes.

We introduce here differential linear scan (micro)voltammetry, DLSV: a novel analytical technique that combines the working principles of linear scan voltammetry, LSV, and the underlying concepts of the numerous existing pulsed voltammetry techniques. DLSV preserves complete and highly accurate information of the electrochemical processes that LSV, due to continuous interrogation in voltage, inherently provides, as well as the much better selectivity and sensitivity of pulsed differential techniques. DLSV also minimizes the background current to negligible levels relative to both LSV and pulsed voltammetry. The practical elimination of background is especially important in biological media where due to the presence of molecules that tend to adsorb onto solid electrodes, the background current is often comparable to, or even larger than the current that carries the actual information of interest in both LSV and pulsed techniques. This problem is even more pronounced with microelectrodes, and/or in media with high concentrations of live cells and because of the complex composition of the required media itself.

DLSV has been tested in this work in challenging experimental contexts: measurement of oxygen using kinetically slow oxygen reduction at a carbon fiber microelectrode in buffer, and with a gold microdisc electrode exposed to a live biological preparation with high concentration of live cells in the vicinity of the electrode. The results show negligible background and stable calibration.

Keywords: Biomedical, Electrochemistry, Voltammetry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Metalloporphyrin ionophores provide ion-selective electrodes (ISEs) with selectivity by selective binding of the analyte anion at one of their two axial ligation sites. While it has been shown in the inorganic literature that binding of electrically neutral ligands at the second ligation site (i.e., on the opposite side of the porphyrin ring) cannot only modulate the strength of anion binding to the ionophore but also affects the selectivity of anion binding, this effect has to the best of our knowledge not been taken advantage of to control the selectivity of ISE membranes doped with metalloporphyrin ionophores.

This talk will introduce this principle and show experimental evidence for this effect using the ionophore Zn(II) tetraphenylporphyrin, which has recently been shown to exhibit a high selectivity for cyanide [1].

Reference:

Keywords: Clinical Chemistry, Environmental, Ion Selective Electrodes
Application Code: Clinical/Toxicology
Methodology Code: Electrochemistry
A new type of squaramide-based tripodal derivatives was studied as ionophores for the fabrication of polymeric membrane anion-selective electrodes. These compounds showed high binding constant with sulfate as well as the good selectivity in solution phase. Various membrane compositions were evaluated systematically to optimize the anion carriers 1-3, the type of plasticizers and the concentration of lipophilic additive to give rise to the optimal sulfate sensitivity and selectivity. The electrodes prepared with membranes containing 0.8 wt % ionophore 2 and 50 mol % TDMACl plasticized with 2-Fluoro-2' -nitrodiphenyl ether demonstrated a Nernstian response of -28.1 mV/decade toward sulfate in the concentration range from 10^-6 to 10^-2 M with significantly enhanced selectivity in comparison to the ionophore free membrane. Moreover, the electrodes exhibited a fast response time and good stability.

Keywords: Biomedical, Environmental Analysis, Ion Selective Electrodes, Sensors
Application Code: Environmental
Methodology Code: Sensors
Nanobiodevices based on advanced nanotechnology open up a novel research field for biomolecule analysis with the ultrahigh resolution, including a single biomolecule analysis. An electrophoretic operation of biomolecules by utilizing artificial nanostructures in microchannels has emerged as a promising technique since it was first proposed. A number of unique artificial nanostructures, such as nanopillar arrays [1] and nanowall arrays [2] have been examined to control the dynamics of biomolecules. These highly ordered nanostructures have provided less time-intensive and required fewer manual operations when compared with other conventional methods. Although recent progresses in micro- and nano-fabrication technologies have allowed us to fabricate smaller and more precise artificial nanostructures for manipulating biomolecules, there is still an inherent size limitation of lithographic technology.

Self-assembled one-dimensional nanowires down to several nano-meters have attracted much attention due to not only the fundamental interests in nanoscale-confined properties but also novel nano-device applications, where existing nanomaterials have not been applicable. Among various nanowire materials, “oxide nanowire” is most promising candidate for the artificial nanostructures, due to their robust surface properties and fascinating redox surface events especially contacting with water, which are hardly attainable to other conventional semiconductor nanowires. In this talk, we demonstrate the feasibility of bottom-up nanowire array embedded in microchannels using vapor-liquid-solid (VLS) methodology, and the application example for biomolecule analysis [3].


Keywords: Bioanalytical, Electrophoresis, Lab-on-a-Chip/Microfluidics, Nanotechnology

Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
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**Abstract Text**

To understand protein function, sequence and structure determination offer essential information for drug design and understanding of structural biology. However, determination of crystallization condition needs trial error, and therefore takes long time and efforts. Several techniques have been developed to overcome this problem. Microfluidic technology based platforms have already been reported as convenient tools to explore for protein crystallization condition. Furthermore, another application for protein crystal structure analysis, such as in-situ X-ray diffraction measurement, decoupling of crystal nucleation and growth, and membrane protein crystallization techniques are also developed using microfluidic format. However, the most important step, nucleation and crystal growth behavior of protein crystal in a microspace, is not yet investigated in detail. To elucidate these phenomena of protein crystallization in microspace provides significant information for controlling crystal nucleation and crystal growth to obtain large single crystal.

We have been analyzed crystal growth behavior in microfluidic format, and elucidate that the crystal growth mechanism rely on the diffusion of protein molecules. Based on this finding, we could control crystal growth by the control of the dimension of the space. We also developed in-situ analytical technique to observe protein crystal growth using confocal Raman spectroscopy analysis. Efforts to control nucleation step to accelerate crystal formation will also be reported.

**Keywords:** Bioanalytical, Biotechnology, Lab-on-a-Chip/Microfluidics, Protein

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
We are going to report development status of fully automated measuring system of not only inter-molecular endpoint reaction but dynamic interaction for medical diagnosis and food inspection. Our system is highly reproducible, real time data acquisition in a short time and needed no special operator with no probe labeling based on Surface Plasmon Resonance (SPR).

This fully automated and stable system is applicable to
1. Analyzing real-time dynamic association/dissociation process, for example, to be able to screening optimum antibody.
2. Antibody or food additive concentration analysis including diluted solution adjustment for calibration curves.
3. Preparation of bio-sensor chip to be optimally modified on chip surface.
4. Regular or specifically cleaning of micro channel with both Aquarius and organic solvent such as ethanol.

In addition to automated measuring system, key technologies of chip manufacturing such as surface treatment, exposure and bonding by VUV light are adopted. These integrated system performance will be shown, and immunosensing for medical diagnosis and food inspection will be discussed.

Keywords: Analysis, Biosensors, Flow Injection Analysis, Process Analytical Chemistry
Application Code: Bioanalytical
Methodology Code: Process Analytical Techniques
The fabrication of functional micro- and nano-particles, such as liposome, quantum dot, gel particle, etc., using microfluidic devices has several advantages over conventional batch methods. For example, the size controlled synthesis of particles can be easily realized by controlling the experimental conditions.

We have developed several types of fabrication method of the multifunctional envelope-type of nanodevices (MEND) for drug delivery system (DDS) using microfluidic devices1-3. Recently, we report the reliable fabrication of MEND using a microfluidic device that had an array of a staggered chaotic mixer with bas-relief structures on the floor of its reaction channel3. The chaotic mixer array allowed homogeneous precursor mixing and that led to reliable results in fabricating MEND. These results should greatly contribute to the kinds development of fast, simple, convenient, economical, and reliable methods for fabricating various of elegant liposome-based non-viral vectors that are suitable for practical gene therapy applications.


Keywords: Biomedical, Biotechnology, Lab-on-a-Chip/Microfluidics, Sample Preparation
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Amino acids play an important role in biological and life sciences. Darrel H. Spackman built amino acid analyzer by ion-exchange column chromatography on an automated apparatus with ninhydrin reagent at the request his mentors (Moore and Stein). This apparatus, which has approximately 40 year history, provides accurate amino acid analysis. The long analytical time and sensitivity are the only drawback in this method.

Recently, liquid chromatography mass spectrometry (LC/MS) has become an attractive tool for not only high-throughput but also highly sensitive and selective analysis. It has been widely used in various fields including life science.

In our work, we explored derivatization reagent to enhance the sensitivity and separation of amino acids by LC/MS, optimized LC/MS condition for high-through put analysis, and developed commercially-based amino acid analyzer (UF-Amino Station). Using this analyzer, 35 kinds of amines and amino acids could be quantified within 12 minutes per sample. There are 3 major factors behind the success of our work. The first is to design suitable derivatization reagents for the amino acid analysis by LC/MS: These reagents has three notable characteristics of sufficient hydrophobicity to enable the retention of amino acids, a desirable structure to increase ionization efficiency, and characteristic and selective cleavage at the bonding site between the reagent moiety and the amino acid. The second is to separate amino acids of the same molar weight on a column in a short analytical time. To achieve high-through put analysis, LC condition was optimized. The third is to build automated pre-column derivatization system for amino acid analysis. This system is extremely users friendly and easy to operate. We expect that a next generation amino acids analyzer is applied for the analysis of amino acids in various fields such as food, agriculture, biochemistry, and clinical diagnostics.

Keywords: Amino Acids, Liquid Chromatography/Mass Spectroscopy
Application Code: Clinical/Toxicology
Methodology Code: Liquid Chromatography/Mass Spectrometry
Advances in optical and scanning probe microscopes have made it possible to watch and manipulate individual biological molecules in action. Just by watching and making the movies of single molecules, we can learn a lot about their dynamic properties. The most successful examples of this approach are linear and rotary molecular motors made of proteins. Motor proteins generate mechanical forces that drive their movements from the energy of chemical reaction. Motor proteins move fast, precisely and efficiently. With single-molecule imaging, the detail of the movements, steps, pauses and conformational changes of motor proteins has been resolved. Furthermore, single-molecule manipulation gives insights on the mechanisms of energy conversion and force generation. Our efforts have helped much to understand the operation principles supporting sophisticated behaviors of motor proteins. Furthermore, by utilizing the insights gained from the biological molecular motors, we have started to create hybrid or artificial molecular motors. The materials used are biological or non-biological molecules, or hybrid of both. Although this approach is still in an early stage, we have succeeded in creation of a hybrid molecular motor and observed its motion. I believe that watching and manipulating molecules one at a time is a powerful approach and will lead to a delightful, exciting future of biology and chemistry. Above all things, they are fun! I’m really happy if you also enjoy the molecular movies in my talk.
Analytical methods capable of quantifying proteins in a single cell are needed in cell biology and medical science research. Miniaturized immunoassays that use ultra-small femtoliter to attoliter sample volumes such as the extended nanospace, can satisfy this analytical need. However, selective capture of analytes at defined positions through antibody surface patterning of nanochannels is challenging. In the present study, we developed a novel antibody patterning method using a photolithographic technique with vacuum ultraviolet light and low-temperature (100°C) bonding. The method enables patterning of functional groups for antibody immobilization before bonding. An immunochemical reaction space with a volume of 86 fL was constructed. Reaction rate analyses indicated that the required sample volume was decreased to 810 fL, and the limit of detection was improved to 3 zmol, 5-6 orders of magnitude better than the microfluidic immunoassay format. High capture efficiency without loss in the small reaction space is highly advantageous for the analysis of low-abundance analytes.

Keywords: UV-VIS Absorbance/Luminescence
Application Code: Bioanalytical
Methodology Code: UV/VIS
Visible spectrometer system designs have evolved rapidly over the last few decades after a major paradigm shift occurred as spectroscopy systems advanced from bulky lab based instruments to the modern compact, flexible, and portable instruments we see today.

The development of low-cost detectors developed for other high volume commercial applications became a major driver for the adoption of spectroscopy in many new fields of science. These systems were made affordable and more pervasive by leveraging the low cost CCDs from bar code scanners and fax machines. Over time, the performance and benefits of these compact systems have improved, blurring the line between high-end scientific systems and these low cost portable systems.

A new era of spectroscopy is around the corner as the recent development of low cost linear CMOS sensors and 2-D imagers combined with extremely powerful compact microprocessors is enabling a new era of even more compact point and imaging spectroscopy systems.

This talk will cover the drivers and features of some of the past systems and discuss how the landscape will change in the next decade as advances in CMOS sensors and portable computing completely changes the opportunities and markets for spectra sensing based systems.

The talk will also discover the roadblocks to a similar growth in the NIR region due to the lack of similar low cost solutions in the 1000+ nm region. New and innovative ideas and solutions to overcoming these challenges with MEMS based technologies will be discussed.

**Keywords:** Near Infrared, Spectrometer, Spectroscopy, UV-VIS Absorbance/Luminescence

**Application Code:** General Interest

**Methodology Code:** Near Infrared
Spectroscopy for Everyone – Smaller, Cheaper, in the Field

Bringing High Field NMR Methods onto the Lab Bench with a Compact NMR Spectrometer

NMR is one of the most powerful analytical techniques due in part to modern multidimensional and multinuclear NMR methods. These typically two-dimensional routine methods allow complex and detailed molecular spectral information to be revealed as couplings between 1D NMR spectra. However until recently these methods have largely been restricted to NMR systems with large, expensive and fragile high field superconducting magnets which cannot be situated on the bench in a chemistry lab.

We report here on a new class of compact, benchtop NMR spectrometer that can not only perform standard 1D NMR experiments, but can also perform more complex and useful 2D NMR methods. Brining NMR into the chemistry lab opens the door to using this powerful, non-destructive, quantitative and powerful analytical technique in a wide range of applications from reaction monitoring, screening, education, and routine analysis.

Keywords: Instrumentation, Magnetic Resonance, NMR, Portable Instruments
Application Code: Other (Specify)
Methodology Code: Magnetic Resonance
Near-infrared (NIR) spectroscopy is a well established non-destructive qualitative and quantitative analysis technique which has been widely deployed in various control laboratories and in manufacturing processes. In an effort to optimize productivity, rather than continuing down the road sending samples to laboratory for analysis, the industry is choosing to change course and adopt portable instrumentation for on-the-spot analysis.

We will present the implementation of portable handheld NIR analysis for on-spot quality monitoring for Feed, food and other applications. Applications are available to rapidly measure multiple quantitative parameters such as protein, fat, moisture, starch, etc. in-field or in production environment. Further applications such as rapid “in-field” detection of Asbestos in various construction materials will be presented.

Technical issues and advantages of Thermo handheld NIR Analyzers, its software will be shown.

Keywords: Agricultural, Food Science, Near Infrared, Portable Instruments
Application Code: Agriculture
Methodology Code: Portable Instruments
A common perception is that mass spectrometry involves bulky, complex, and expensive equipment requiring frequent and costly maintenance. Additionally, many chemists and other users find that queued access to communal instruments disrupts work-flow and is incongruous with many potential applications. Microelectromechanical systems (MEMS) technology has enabled Microsaic Systems to develop an affordable, miniature instrument that can be readily deployed as a general purpose detector at the bench or in the field. The 4000 MiD is contained within a single enclosure and does not require an external backing pump or separate computer. The instrument is controlled using intuitive software, and is easily set-up and maintained by non-specialists. The core technology and ongoing efforts to develop new applications will be described.
In 2013 Pie Photonics brought its first static optical fiber interferometers to the market place and won a Best New Technology Award at Pittcon. Pie interferometers are portable, broadband, high-resolution instruments that deliver the power of interferometry and FT-spectrometry to a wide range of optical metrology and spectroscopy applications in the field, on production lines and at point of care, via a single-engine, no-moving parts design. With access to wavelength, phase, delay and amplitude information in the temporal and spectral domains, Pie interferometers are configured to measure wavelengths with sub-nanometer resolutions over super-octave ranges, detect wavelength shifts with picometer accuracy and time attosecond delay changes. Initial research into monolithic photonic engine solutions based on photonic crystal and multi-core fiber circuit designs has proven the possibility for ultra-compact, lightweight and low-footprint solutions that can truly mobilize the power of interferometry and bring its power to mass market applications.
Defiant Technologies has developed two “lab-on-a-chip” based systems for portable chemical analysis. Defiant uses micro electromechanical systems (MEMS) fabrication processes as well as designer nanomaterials to create very small and low power components like our micro preconcentrator and micro GC column. The first portable instrument called the Canary-3 is a handheld GC/SAW system made for the detection of chemical warfare agents in air. The chemistry applied to the micro preconcentrator makes it possible to detect low concentrations of CWAs even in the presence of high concentrations of interfering chemicals like diesel fumes. In addition, the Canary-3 can intelligently collect target chemicals making it difficult to overwhelm the system with a high concentration. The second portable instrument is a purge and trap micro gas chromatograph (GC) system specifically designed for detection of volatile organic compounds (VOCs) in water, soil, and air. The system dubbed the FROG-4000 is capable of detecting VOCs like benzene and trichloroethene at 0.5 ppb in water. Both the Canary and the FROG weigh less than 5 pounds. Each scrub ambient air for the carrier gas and each will operate on rechargeable batteries for 6 to 8 hours. Each can be operated with a computer to view data live or they can be used standalone in the field and report chemical name and concentration to their displays. In our presentation, we will discuss the components, their operation, and how they are integrated into a system. In addition, we will discuss smart sample collection, results from field tests with split samples, and results from certified reference materials.

Keywords: Gas Chromatography, Lab-on-a-Chip/Microfluidics, Portable Instruments, Volatile Organic Compound
Application Code: Environmental
Methodology Code: Microfluidics/Lab-on-a-Chip
This paper describes progress towards developing chip-scale integrated optic miniature gas sensors based Tunable Diode Laser Absorption Spectroscopy. We demonstrate the practicality and feasibility of measuring methane in sensor packages of a few cm³ at costs suitable for very high volume production. The sensors will find application in networks for environmental monitoring and natural gas leak detection.
Air sampling is a key factor in the environment which influences human health and well-being. Many environmental pollutants induce toxic, mutagenic and carcinogenic effects in living organisms. Subsurface vapor intrusion to indoor air is a potential pathway of human exposure and health risks for sites with volatile organic compounds in soil and groundwater. Consequently, a number of regulations regarding maximum permissible concentration level of the pollutants were established in the recent years due to growing environmental concerns. For years the focus of the research on vapor intrusion was directed towards the development of cheap, easy, effective and quantitative ways of vapor sampling. Therefore, the last decade abounds in many different methods for air quality sampling and assessment. The major disadvantage of many methods developed so far is that they are laborious and time-consuming. One method that overcomes the drawbacks of conventional sampling methods is passive sampling, both equilibrium and kinetic. The popularity of passive sampling is due to its low cost, simple deployment, minimum training required and lack of external power sources needed. Passive sampling is increasingly often used in vapor intrusion studies for contamination mapping and for effective soil remediation.

This work presents an overview, advantages, limitations and applications of polydimethylsiloxane (PDMS)-based passive samplers developed at the University of Waterloo. The hydrophobicity of PDMS allows them to be exposed for extended periods of time to humid atmospheres. The time-weighted average (TWA) concentrations determined by the samplers are better estimates of long-term average concentrations than short-term concentrations determined using active sampling. The results are characterized by good accuracy and precision. Applications of PDMS-based samplers to soil gas analysis will be discussed in detail.

Keywords: Environmental, Environmental/Soils, GC-MS, Sample Preparation
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
The ability to rapidly detect and identify trace hazardous analytes in air has become increasingly important for including industrial hygiene, battlefield operations, and environmental contamination. It is not uncommon for a trace level analyte to cause a major health problem. Conventional air samplers typically sample air at a flow rate of 100-200 mL/min. With 10 ng/m$^3$ concentration, a conventional sampler would require ~100 L and ~500 min to sample. To reduce the time for reliable quantitation, we have developed a high flow system that can sample up to 10 L/min, reducing the sampling time by 100-fold. The system consists of a uniquely designed concentric high flow rate trap connected to a micro concentrator, which can be interfaced to the GC-MS. With our high flow air sampling system, a 10 ng/m$^3$ sample can be collected in 10 min and transferred to the GC-MS in a 1.5-s narrow band. By reducing the sampling time, not only is the analysis time conserved but personnel are less exposed to potential toxic compounds, and critical decisions can be made quickly. Funding from the US Department of Defense through subcontracts from Smith Detection and Torion Technology is gratefully acknowledged.

Keywords: Capillary GC, Quantitative, Sampling, Thermal Desorption
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Time-Weighted Average Sampling of Volatile Airborne Organic Compounds by Needle Trap Devices (NTD)

The commercially SGE Needle Trap Devices (NTD) can be used as a diffusive passive sampler for volatile compounds. The NTs with side hole above the sorbent bed and an extended tip were produced by SGE Analytical Science (Victoria, Australia). NT is an exhaustive, solvent less and one-step sample preparation technique that can be easily calibrated. The use of NTs for Time-weighted average sampling (TWA) results in many advantages over traditional sampling methods. This approach eliminates errors of sample transportation. It also reduces analysis time and improves accuracy and precision in analytical results. Passive sampling provided a reliable method of analyzing low concentrations of volatile air contaminants in short time. The basic principle of passive sampler relies on diffusion to move the analytes from matrix contained in tip of needle to the extraction phase. The standard gas generator system was employed for the evaluation of needles in passive sampling. The standard permeation tubes were filled with benzene, toluene and ethyl benzene compounds and placed in permeation chamber at constant temperature and Nitrogen flow. The NT samplers were placed in sampling chamber for 60, 90 and 120 min to determine the concentration of target compounds with diffusive sampling method. There is a good agreement between the theoretical and experimental sampling data. Indoor air was analyzed in a chemistry laboratory at the University of Waterloo. Several samples were collected in the span of a workday (8 h). Active sampling through a NTD was carried out every hour to observe the intra-day variations. Passive sampling over a period of 8 hours was used to determine the average concentration of toluene that workers were exposed to. Good agreement was observed between passive and active techniques.

Keywords: GC, GC-MS, Trace Analysis, Volatile Organic Compounds
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Air Sampling for Environmental Applications (Half Session)

Pollutant Source Attribution Using Wireless Air Quality Networks

Recently published results from air quality networks in Cambridge UK (Atmospheric Environment 70 (2013) 186-203) showed how the use of low cost mobile monitors and fixed site motes could be used to locate and quantify pollution “hot spots”.

A more recent project, using a high density wireless air quality network at Heathrow airport employs low cost sensors to monitor inorganic gases, VOCs, particulates/aerosols and wind speed/wind direction continuously. This combination of air quality measurements allows source attribution that is not possible with individual monitoring sites.

We will show how combined measurements, along with knowledge of wind direction can be used to distinguish aircraft emissions (including differentiating the LTO cycle from taxiing) from automotive emissions and background pollution from local sources. As an example, the plots below show bivariate source attribution plots (concentration vs wind speed and direction) for carbon monoxide and nitric oxide in the vicinity of the southern Heathrow runway, in this case showing elevated levels with wind in the north-easterly direction (the direction of the runway).

We conclude that state of the art low cost sensors, coupled with suitable analysis and interpretation software are now useful tools for 24/7 tracking of potential pollutants at industrial sites, during construction programs and emergencies.

The authors are grateful for the support of the UK Natural Environment Research Council, Heathrow Airport Limited and British Airways. Bivariate plot software courtesy of openair (www.openair-project.org).

Keywords: Electrochemistry, Environmental/Air, Integrated Sensor Systems, Sensors

Application Code: Environmental

Methodology Code: Integrated Sensor Systems
Electrochemical cytometry is a technique based on the electrophoresis of bioparticles with amperometric detection of zeptomoles of material encapsulated within a subcellular organelle. Rat pheochromocytoma cells (PC12) are known to secrete catecholamine neurotransmitters which can be monitored directly upon release using single cell amperometry. The data obtained by single cell measurements are completed by data obtained from electrochemical cytometry experiments because of the high-throughput nature (1000’s of vesicles analyzed) of the cytometry experiment. Advances in electrode stability, noise reduction, and capillary preparation will allow for routine analysis of PC12 secretory granules. Because of the nature of electrochemical cytometry, relatively crude preparations of secretory granules can be studied and quantitative information on catecholamine content and electrophoretic mobility can be obtained. This eliminates the need for ultracentrifugation and size exclusion chromatography and greatly reduces the cost and time for preparation of secretory vesicles.
Embryonic stem cells (ESCs) are specialized cells that have the potential to be utilized in many medical and therapeutic applications. However, before these applications can be pursued further, the techniques required for a successful large-scale culture and expansion of undifferentiated ESCs remain under development. Currently, feeder cells, which provide essential nutrients to ESCs, are growth-arrested by chemicals or gamma (\gamma) irradiation. Both of these techniques have limitations that can affect the ESCs. In this study, we utilized pulsed electric fields (PEFs) to generate growth-arrested feeder cells for the successful culture of undifferentiated mouse ESCs (mESCs). We found that the feeder cells remained growth-arrested and viable when exposed to a given electric field. Furthermore, when mESCs were seeded on feeder cells exposed to PEFs, they remained undifferentiated through several sub-cultures and were able to differentiate into functional cardiomyocytes. Therefore, PEFs provide an effective and inexpensive new technique to prepare feeder cells for a successful culture of pluripotent ESCs.
Amperometric nitric oxide (NO) sensors are sensitive, compact, and inexpensive devices that are capable of measuring NO directly in physiological systems. While existing NO sensors typically exhibit high selectivity over non-volatile electro-active species, volatile species such as carbon monoxide (CO) remain problematic. Indeed, measurement of NO in exhaled nasal breath is a potential diagnostic for assessing risk of sinus infection and other respiratory conditions, but CO levels in nasal breath are quite high, making it rather challenging. Cyclic voltammetry reveals that formation of an oxidized Pt film (on Pt working electrode) at the anodic potentials used to polarize NO sensors inhibits adsorption of CO onto the electrode surface, which is necessary for electro-oxidation of CO on Pt. Previous amperometric NO sensors that employ inner electrolyte solutions have utilized acidic internal solutions, which inhibit the formation of a dense platinum oxide films. In this presentation, we will show that by increasing the internal electrolyte pH within NO sensors to alkaline conditions, platinum oxide film formation is promoted, and this results in up to ~100-fold increase in selectivity over CO with LOD of <100 ppb when the sensors are operated in the gas phase, and 1 nM when operated in solution.
Carbon fiber microelectrodes (CFMEs) have been used for many years for the detection of neurotransmitters using fast scan cyclic voltammetry. However, more sensitive, selective, and faster electrodes are sought to further enhance the detection of neurotransmitters. This has primarily been attempted through the use of carbon nanotube based electrode technology. Carbon nanotube (CNT) based electrodes have been used to increase conductivity, have larger electroactive surface areas than carbon fibers, and resist analyte surface fouling. CFMEs dip-coated with CNTs have caused increases in noise and lack reproducibility. Wet spinning CNTs into fibers provides a thin uniform fiber that can be used as an electrode. CNTs were separated using a surfactant and wet spun into a polymer solution that collapsed CNTs into ribbons that were purified into fibers in methanol. CNT fibers were epoxy insulated and silver epoxied to a gold pin to form electrodes. CNT fiber electrodes exhibited lower limits of detection, 5 nM, with respect to CFMEs. A resistance to surface fouling was observed when detecting analytes such as serotonin that can form a polymer at the surface of the electrode and hence decrease sensitivity. Sensitivity was frequency independent with CNT fiber electrodes. This allows for faster measurements at higher frequencies, which could be conducted on the time scale at which neurons fire, up to 500 Hz. In the future, we will also develop additional methods of CNT fiber construction such as wet spinning with acids instead of polymers. Carbon Nanotube fibers can be made into electrodes that have many superior qualities with respect to CFMEs that can detect dopamine at lower concentrations and on a faster timescale.

Funded by the NSF CHE0645587522

Keywords: Bioanalytical, Electrode Surfaces, Nanotechnology, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Dysfunctions in the brain’s serotonergic system have been implicated in many neuropsychiatric disorders including depression, schizophrenia, obsessive compulsive, and anxiety disorders. Therefore, serotonin neurochemistry is an important target for study. We use fast scan cyclic voltammetry in mice to examine serotonin neurotransmission. Three distinct, heterogeneous serotonin responses to a similar electrical stimulation were observed: slow, fast and hybrid, as seen previously for dopamine(1). Mathematically modeling these responses yielded separate transporter efficiencies (Vmax) for the slow and fast signals. The fast responses showed higher transporter efficiency, whereas the slow showed lower efficiency. The hybrid responses, when modeled, have a combination of the two efficiencies. This implies there are two separate serotonin uptake mechanisms, which may give clues to the pathophysiology and treatment of the disorders in which this monoamine is implicated.


Keywords: Microelectrode, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
In this presentation we will describe the use of chemically- and electrochemically-synthesized metallic nanostructures for spectroscopic, electronic, or combined spectroscopic and electronic detection of various vapor phase or solution phase analytes. We first focus on microelectrodes that have Ag nanowires or Ag nanoparticle arrays across them. The junctions display on/off electronic switching behavior and surface enhanced Raman scattering with organic molecules or thin films as part of the metal/organic/metal junction. The electronic properties and Raman scattering measurements correlate with one another and can serve as a platform for dual detection of various analytes of interest. We also focus on the synthesis of Au nanoplate/Au nanoparticle junctions for detection of analyte by localized surface plasmon resonance spectroscopy and surface enhanced Raman spectroscopy at the single particle level. Sensitivity is improved by controllably placing nanoparticles on the edge sites of nanoplates as an amplification strategy. The effect of nanoparticle location on signal amplification will be discussed.

Keywords: Electrochemistry, Metals, Nanotechnology, Surface Enhanced Raman
Application Code: Nanotechnology
Methodology Code: Electrochemistry
Shewanella oneidensis bacteria have been noted for its potential use in clean energy production and water purification due to its capability to metabolize a variety of species, including insoluble metal oxides and toxic metal species. This ability to transfer electrons to a material that cannot freely diffuse through the cell membrane is known as dissimilarity metal reduction (DMR). Because the DMR process generates a current on a variety of electrodes, previous work has electrochemically elucidated the mechanism by which DMR occurs in S. oneidensis. While several mechanisms have been proposed for this process, S. oneidensis demonstrates a greater reliance on soluble electron shuttles, in the form of flavins, to perform DMR. Although S. oneidensis biofilm DMR capabilities have been investigated, there is little information on the production and consumption of electron shuttles for small clusters or individual bacterial cells.

In this research, scanning electrochemical microscopy (SECM) is used to detect the current generated from the DMR process. Coupling sub-micron tipped ultramicroelectrodes (UMEs) with the high spatial resolution provided by SECM allows for the imaging of small clusters and single bacteria through the real time monitoring of electron mediator flux. This technique provides valuable information on the reactivity and topography of bacteria on solid substrates, enabling investigations into the differing DMR pathways of individual S. oneidensis cells, as well as production and consumption sites of flavins.

This understanding of the DMR mechanisms is necessary in the optimization for use of S. oneidensis in bioenergy and bioremediation applications. Furthermore, the use of SECM with UMEs to study small clusters and single cells provides an additional tool for analytical research on single cell organisms.

**Abstract Text**

Bioanalytical Electrochemistry: Assorted Applications and Methods

**High-Resolution Scanning Electrochemical Microscopy (SECM) Studies of Dissimilarity Metal Reduction Pathways of [i]Shewanella Oneidensis[/i]**

**Abstract Title**

[i]Shewanella oneidensis[/i] bacteria have been noted for its potential use in clean energy production and water purification due to its capability to metabolize a variety of species, including insoluble metal oxides and toxic metal species. This ability to transfer electrons to a material that cannot freely diffuse through the cell membrane is known as dissimilarity metal reduction (DMR). Because the DMR process generates a current on a variety of electrodes, previous work has electrochemically elucidated the mechanism by which DMR occurs in [i]S. oneidensis[/i]. While several mechanisms have been proposed for this process, [i]S. oneidensis[/i] demonstrates a greater reliance on soluble electron shuttles, in the form of flavins, to perform DMR. Although [i]S. oneidensis[/i] biofilm DMR capabilities have been investigated, there is little information on the production and consumption of electron shuttles for small clusters or individual bacterial cells.

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This understanding of the DMR mechanisms is necessary in the optimization for use of [i]S. oneidensis[/i] in bioenergy and bioremediation applications. Furthermore, the use of SECM with UMEs to study small clusters and single cells provides an additional tool for analytical research on single cell organisms.

**Keywords:** Biological Samples, Electrochemistry, Microelectrode, Spectroscopy

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
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 to each other. 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 peptide separations. µFFE provides a unique opportunity to greatly improve the performance capabilities of a two-dimensional separation. 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 tryptic peptides of BSA using reversed-phase LC and various µFFE conditions. Peak capacities of up to 2000 have been achieved in 30 minutes.

This work was supported by NSF-CHE Grant #1152022.
The analysis of biological samples is becoming increasingly challenging as researchers from a variety of disciplines seek to simultaneously characterize numerous analytes at the molecular level. The continued expansion of many -omics applications serves as a salient example of this trend. Increasing the scope of biological research necessitates improvements to existing separations methods, which are quickly becoming a bottleneck in sample analysis. One promising approach for dramatically increasing separation performance is to perform multi-dimensional separations, coupling orthogonal separation methods sequentially. Our laboratory has recently developed a two-dimensional separation platform based on coupling capillary liquid chromatography (LC) with microchip capillary electrophoresis-electrospray ionization (CE-ESI). This device is capable of performing rapid separations with high resolving power; achieving peak capacities of over 1400 in 50 minutes for a peptide digest mixture. However, the sample transfer between the LC and CE dimension impacts the sensitivity of this method, as a very low percentage of the LC effluent reaches the CE separation channel. This presentation will focus on the continued development of this technology through the integration of a sample-focusing step between the LC and CE dimensions, increasing the percentage of the LC effluent that reaches the CE channel while maintaining separation performance. This integration improves the sensitivity of the method by at least an order of magnitude, and results in improved sequence coverage and detection of low abundance components.

**Keywords:** Capillary Electrophoresis, Capillary LC, Mass Spectrometry, Separation Sciences

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

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Aspergillus, or black fungus, has a 90% mortality rate for immunocompromised and critically ill patients and if left untreated can kill within a few days. [1] The most prevalent detection methods are cell cultures, antibody assays, imaging procedures, and biopsies, which can be time consuming and/or lack species determination. [2] Therefore, it is imperative to create a simple and rapid device for Aspergillus detection. The use of a microfluidic device with a phospholipid nanogel matrix provides a rapid means of detecting amplified DNA fragments unique to Aspergillus. In conventional gel capillary electrophoresis, phospholipid nanogels have aided in the separation of short tandem repeats of DNA biomarkers, generating from 500,000 to 1,000,000 theoretical plates. [3] This non-Newtonian thermally reversible nanogel, enters the channel as a liquid and gels in-channel with an increase in temperature. This unique property allows for quick removal and replacement of the separation matrix in the microfluidic channel. [4] This talk discusses the advances made in integrating nanogels into microfluidic channels for the separation of biomolecules.

References:
3. Durney, B.C., Archer-Hartmann, S., Holland, L. A., Manuscript Accepted

Keywords: Analysis, Bioanalytical, Capillary Electrophoresis, Nanotechnology
Application Code: Clinical/Toxicology
Methodology Code: Capillary Electrophoresis
The determination of morphine and its isobaric metabolites morphine-3-beta-D-glucuronide (M3G) and morphine-6-beta-D-glucuronide (M6G) is useful for therapeutic drug monitoring and forensic identification of drug use. In particular, capillary electrophoresis with mass spectrometry (CE-MS) represents an attractive tool for opioid analysis. Whereas volatile background electrolytes in CE will improve electrospray ionization for coupled MS detection, such electrolytes may reduce CE separation efficiency and resolution. To better understand the effects of background electrolyte (BGE) composition on separation efficiency and detection sensitivity, this work compares and contrasts method development for both volatile (ammonium formate and acetate) and nonvolatile (ammonium phosphate and borate) buffers. Peak efficiencies and migration times for morphine and morphine metabolites were optimal in a 25 mM ammonium borate buffer (pH=9.5) although greater sensitivities were achieved in the ammonium formate buffer. The coupling of optimized CE methods to high mass accuracy time of flight (TOF) MS detection allowed for the resolution of the isobaric morphine metabolites in less than seven minutes. Urine sample preparation required only a 10-fold dilution with BGE. Limits of quantitation in normal human urine were found to be 1.0 [micro]g/mL for morphine and 2.5 [micro]g/mL for each of M3G and M6G. These LOQs were comparable to those for CE-UV analysis of opioid standards in buffer, whereas CE-TOF-MS analysis of opioid standards in buffer yielded LOQs an order of magnitude lower. Patient samples (N=12) were analyzed by this new CE-TOF-MS method and a 7% difference in total morphine content (relative to prior LC-MS results) was found. The LC-MSMS method used for these data followed enzymatic hydrolysis of the glucuronides and hence was only able to determine the total morphine species concentration. This new CE-TOF-MS method allowed for species differentiation in addition to total morphine determination. By this method, it was found that M3G and M6G metabolites were present in a 5:1 concentration ratio in each of the patient samples. Therefore, the CE-TOF-MS method not only allows for total morphine concentration determination comparable to established LC-MSMS methods, but also allows for differentiation between morphine and its glucuronides, yielding additional biochemical information about drug metabolism.
A fast liquid-liquid extraction using a hydrophobic surfactant-based room temperature ionic liquid (RTIL), coupled with capillary electrophoresis was developed for the extraction and quantitative determination of phenolic compounds. This was possible because the RTIL used was insoluble in water and therefore was used in place of volatile organic solvents. In addition, the anions derived from the surfactant part of the RTIL may reduce the interfacial energy of the two immiscible liquid phases, resulting in efficient extraction of analytes. Parameters such as nature of ionic liquids, pH of aqueous solution, extraction time, and the presence of salt in aqueous solutions were investigated. In this regard, acidic conditions allowed extraction of most of the investigated phenols from aqueous solution into RTIL phase within the shortest time. Finally, a simple and fast detection method was developed to separate and determine the extracts on account of the selection of the RTIL. Validation of the developed method was achieved via cleanup and analysis of spiked standards in actual water samples.

Keywords: Capillary Electrophoresis, Extraction, UV-VIS Absorbance/Luminescence, Water
Application Code: Environmental
Methodology Code: Capillary Electrophoresis
Capillary and Micro-Free-Flow Electrophoresis

Coupling Micro Free-Flow Electrophoresis with Desorption Electrospray Ionization Mass Spectrometry (DESI-MS) for Proteomic Analysis

Micro free-flow electrophoresis (FFE) is a separation technique that holds promise for the simultaneous purification, concentration, and separation of proteins. In FFE, analyte streams are deflected laterally in a planar flow channel by a perpendicularly applied electrical field. Currently there is no method for introducing samples into a mass spectrometer from a FFE device. We have investigated capturing proteins onto an intermediate membrane as they exit the separation channel of an open-edge FFE device for DESI-MS analysis of the membrane. The potential benefits of this application for proteomics include reduced time and sample complexity, protein capture, concentration, and purification.

In an attempt to couple FFE to DESI-MS, a glass open-edge FFE device was fabricated by typical photolithography and wet etching techniques. The device was cut perpendicularly through the separation channel and a permanent coating of polyvinyl alcohol applied to the microfluidic channels, suppressing electroosmotic flow and protein adsorption. Optimization of the FFE protein separation was done using fluorescently labeled myoglobin and BSA. The DESI source parameters were optimized by desorbing a rhodamine based dye, and myoglobin from glass. After optimization, myoglobin and BSA were captured on membrane after separation in the open-edge FFE device. The captured proteins were characterized by DESI-MS analysis of the membrane. The proteins were successfully separated and captured on membrane for DESI-MS analysis taking less than one hour total for separation, protein capture, and DESI-MS analysis. More complex samples were also explored.

This research was supported by NSF Grant # 1152022.

Keywords: Electrophoresis, Lab-on-a-Chip/Microfluidics, Mass Spectrometry, Protein
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Exposure to particulate matter (PM) air pollution is one of the top-10 leading causes of morbidity and mortality in the world. Public health studies have shown that chronic exposure to PM contributes to cancer, cardiovascular disease, respiratory disease, and premature death. In particular, occupations such as metalworking, construction, transportation, and mining are especially hazardous because inhalation of metal oxides can interfere with numerous intracellular biochemical processes. Although millions of individuals are exposed to metal-containing PM, very few are monitored for their exposure due to the time-intensive and cost-prohibitive analytical methods currently used. To address this concern, we have developed two new paper-based sensors for measuring transition metals (Mn, Cu, Ni) colorimetrically and heavy metals (Zn, Cd, Pb) electrochemically. Our paper sensors are capable of sub-ppm detection limits with analytical costs hundreds of times lower than traditional methods. The devices are simple to operate and do not require bulky instrumentation for analysis. Distance-based quantification is performed solely by visual assessment; a portable, inexpensive potentiostat was built to perform electrochemical measurements. To demonstrate the utility of this device, chemistry was developed for measuring metal content from several environmental sources such as welding fumes and roadside emissions.
Nitrogen oxides are important precursors for ozone formation. O₃ is not only an essential component of the well known photochemical smog frequently forming over highly populated and industrialized areas in summer, it is also an important greenhouse gas with the largest effect in the tropopause region. In urban areas with significant NOx emissions, nitrogen oxides are monitored with standard Chemiluminescence detectors (CLD) with molybdenum converters. But in remote locations and at higher altitudes these measurements require higher sensitivities and accuracies and therefore molybdenum converters for NO₂ determination are not suitable [1]. More accurate converters for remote locations and at high altitudes are photolytic converters (PLC). In ambient measurements there is a substantial amount of O₃, which reacts in the photolysis chamber. Depending on the amount of sunlight, the concentration of O₃ varies daily. For a correct measurement of NOx this substantial influence must be corrected.
In this research, we report the development and field study of a prototype GC to demonstrate real-time analysis of VOC mixtures in a semiconductor process clean room. This prototype consists of a multi-stage preconcentrator, a 6-m capillary column (0.25 mm i.d., PDMS), a photoionization detector unit and a tablet computer. The column temperature can be ramped as fast as 120 deg.C/min for rapid mixture separation via at-column heater. Scrubbed air was used as the carrier gas to eliminate cylinders and reduced overall size. All fluidic and electric interconnections and a tablet computer were integrated inside a metal case that measures 30 (l)x 17 (w)x 8 (h) cm. The software for instrument control and chromatographic data algorithm was written in our group using LabVIEW. Each GC analysis cycle was completed within 15 min. The field analysis using GC was performed continuously during a workday alone with a single canister/GC-MS analysis that served as a reference. There were six compounds identified by GC-MS: acetone, isopropyl alcohol, n-hexane, toluene, m-xylene and anisole in this clean-room. The detection limits of prototype GC were ranged from 0.03 (toluene) to 0.16 (isopropyl alcohol). The concentrations measured by GC varied from 0.3 to 20 ppb which match in range with canister/GCMS result. The GC data reveals the time-dependent concentration trends at low ppb range for each compound. The prototype GC provided a cost and time efficient method for fast assessment of complex VOCs contaminations in a clean room process for semiconductor industry.

Keywords: Capillary GC, Environmental/Air, Volatile Organic Compounds
Application Code: Environmental
Methodology Code: Portable Instruments
Biofuels obtained from pyrolysis of prairie cordgrass can serve as a renewable alternative energy source for petroleum. However, the study of the physical and chemical properties of the pyrolysis products is essential to improve the quality of biofuels by removing the undesirable characteristics in the processes. The pyrolysis products which consist of bio-oil, biochar, and pyrolysis gases can be characterized if separation methods are developed to isolate the various components.

Most pyrolysis products are obtained at a temperature of 600 °C. There is interest in developing fast pyrolysis operating at higher temperatures. The objective of this work is to analyze the products from pyrolysis at 600 °C and 900 °C by an automated fractionation method using accelerated solvent extraction (ASE) and gas chromatography-mass spectrometry (GC-MS). Diethyl ether and methylene chloride were used to separate the water-soluble and water-insoluble fractions of the bio-oils respectively using ASE. The resulting fractions were analyzed based on mass recovery and characterization by GC-MS. The results were comparable for the two different temperatures in the diethyl ether fractions, but differences were noted between the methylene chloride samples. The bio-char will be analyzed for porosity, surface area, and related characteristics.

Keywords: Biofuels, Fuels\Energy\Petrochemical, GC-MS, Pyrolysis
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography/Mass Spectrometry
This work presents and compares two techniques to quantify some VOCs down to ppt level in natural gas, biogas and syngas.

Polycyclic Aromatic Hydrocarbons (PAHs) and Volatile Organic Compounds (VOCs) may occur in natural gas at sub-ppb levels. They also may be found in biomethane (depending on the production sources) and especially in syngas where PAHs are formed during the gasification process. Some of these PAHs and VOCs are known to be carcinogenic, mutagenic and teratogenic. Because of the steadily increasing production of these new gases, the identification and quantification of such compounds are crucial for health and environmental issues.

Two analytical methods were developed to sample and quantify these PAHs and VOCs. Both methods rely on a two-step process: preconcentration during sampling and analytical quantification using a gas chromatograph coupled with mass spectrometer (GC-MS).

The first method consists in a preconcentration on XAD-2 resin followed by liquid extraction with Assisted Solvent Extraction (ASE) and in a GC-MS analysis. The second one is based on a preconcentration step on a Tenax adsorbent followed by an in-line thermodesorption coupled with GC-MS.

We carried out a design of experiments for both steps. It allows us, through statistical tools, to determine the best set of parameters, optimize and validate these methods.

To conclude, both methods were compared regarding their performance: limit of quantification, reproducibility, selectivity and accuracy were determined. The TDS-GC-MS technique showed better performance compared to liquid extraction and is solvent-free and easy to operate.
California recently enacted legislation to mandate monitoring of toxic and carcinogenic compounds in biomethane from landfills, publicly owned treatment works (sewage treatment plants) and dairies prior to allowing this gas to enter the natural gas pipeline within California. The California Public Utilities Commission, in conjunction with the California Air Resources Board and Office of Environmental Health Hazard Assessment, has adopted criteria for acceptance of biomethane for public use. Implementation of these new requirements is set for the end of 2013. These include measurement of a list of Constituents of Concern and proposed test methods. The action levels are based on risk assessment for human health and require adjustments to the suggested protocols to make accurate appraisals of these analytes. Eight of the constituents can be assessed by gas chromatography with mass spectrometric and sulfur detectors.

Lotus Consulting has configured a Bruker SCION/456-GC GCMS with a Pulsed Flame Photometric Detector (PFPD) to measure these eight compounds, and has adapted the reference measurement procedures to handle the specified action levels. A Flame Ionization Detector (FID) is used to provide a rapid screen of concentrations for organics. Based on this result, an appropriate GCMS and PFPD method is automatically selected to perform the measurement. Final results are compared to the mandated trigger and action levels, and a flag is generated for compounds exceeding these levels. The referenced test methods are specifically designed for ambient air measurements and are not set up to handle the significantly higher concentration ranges specified by the new regulations, and a different bulk gas of methane, instead of ambient air. This analyzer modifies these approaches by using fixed volume loops to directly inject samples into the column sets, without requiring tedious serial dilutions to get sample concentrations within range of the reference methods.

Keywords: Analysis, Biofuels, Gas Chromatography/Mass Spectrometry, On-line
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography/Mass Spectrometry
The California Public Utilities Commission has mandated that biomethane must be assayed for a list of “constituents of concern” and has set action levels based solely on risk assessments for human health. Most of the compound action levels for organics are in the medium to high part per million levels. Accurate and stable gas standards are required for proper quantitation of these compounds.

Gas standards in cylinders for these analytes suffer from analyte loss from the very low volatility of the target analytes, from the extremely high mandated concentration action levels, and from interactions with cylinder and regulator interior surfaces. One analyte – n-Nitroso-di-n-propylamine – has a low vapor pressure and cannot remain stable in high pressures of cylinders.

This paper reports on the creation of standards for these target toxic organics from permeation tubes. Advantages include:

- Permeation rates NIST traceable
- Fresh standards on demand
- Gas standards for low vapor pressure components
- Selectable concentration levels by simple adjustment of dilution flows
- Positive identification of peaks by selection of permeation tubes being analyzed
- Standard introduction matching process for samples
- Minimal operator interactions
- Easy addition of new “constituents of concern” into mix

Results using permeation tubes containing pertinent volatile organics are presented illustrating long term stability of generated standards, creation of multi-level calibration curves over a wide dynamic range, and computation of detection limits and linear ranges.

Keywords: Biofuels, Calibration, Gas Chromatography/Mass Spectrometry
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography/Mass Spectrometry
Porous Layer Open Tubular (PLOT) GC columns are widely used in petrochemical and light gas applications. Many applications utilize multiple column techniques with switching valves to back flush or heart-cut to route portions of the sample matrix or analytes to columns of different selectivity to enhance resolution and optimize operation. Compared to Wall Coated Open Tubular (WCOT) GC columns, PLOT columns are subject to phases shedding. Even with the latest in bonded PLOT column technology, care must be taken when using these columns as vibration, reverse flow and pressure changes can cause particles of stationary phases to dislodge from the column wall and flow downstream into switching valves rotors, Capillary Flow Technology (CFT) devices and detectors causing detector spikes, damage to switching valve rotors and in severe cases can cause flow restriction. Any of these instances require system maintenance. New development in PLOT columns with integrated particle trapping technology addresses these issues. Incorporating particle trapping capability on both ends of the column is ideal for valved applications and eliminates the need for attaching separate particle traps (via unions) and in-line filters to protect valve components and detectors for improved instrument uptime and even expanding PLOT column use for routine use with mass spec detectors. The technology enhancement is demonstrated on porous polymer, alumina and molecular sieve PLOT columns. GCMS applications using integrated particle trap PLOT columns such as gas analysis and trace oxygenates in mixed C4 streams analysis are presented.

**Keywords:** Capillary GC, Chemical, Petroleum, Process Analytical Chemistry

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

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Organic monolithic stationary phases offer broad chromatographic selectivity, high porosity, and independent optimization of through-pore and skeleton size in liquid chromatography (LC). However, the chromatographic performance of these organic monoliths has generally not reached the level of particle packed columns. To date, synthetic methods for organic polymer monoliths have not provided sufficient control of pore size distribution and monolith morphology to fully realize their potential chromatographic performance. Studying and characterizing the bed structures of organic monoliths along with factors governing them can provide great insight into their performance. We have been working to improve the performance of biocompatible poly(ethylene glycol) diacrylate (PEGDA) monoliths by using different characterization and polymerization techniques. We have been investigating three dimensional scanning electron microscopy (3-D SEM) for in-situ characterization of these organic monoliths, and correlated their structural parameters with chromatographic performance. The experimental measurements for tortuosity and chromatographic parameters were found to be in agreement with values of structural parameters computed using SEM images. The chromatographic performance of the monoliths was found to improve with reduced through-pore dimensions, improved column homogeneity and reduced tortuosity. To obtain more homogenous structures, we are using living polymerization for monolith fabrication because it has been reported to provide good control over polymerization conditions and resultant polymer structure. A resultant organic polymer monolith has given an unprecedented chromatographic performance of 150,000 plates/m for a non-retained compound.

Keywords: Capillary LC, Characterization, HPLC Columns, Liquid Chromatography

Application Code: Other (Specify)

Methodology Code: Liquid Chromatography
In a recent report, sub-2 µm particles with an interconnected network of 50-150 nm macropores were utilized as stationary phase supports for affinity chromatography. [1] In this experiment, the use of these particles for reversed phase capillary ultra-high pressure liquid chromatography (UHPLC) was investigated. A high pressure flushing system confirmed particle stability up to 3000 bar. Chromatographic analysis showed that columns packed with the macroporous particles gave reduced minimum plate heights under 2, indicating good performance. However, the flow resistance of these columns was significantly higher than expected due to nonporous silica fines produced during particle synthesis. Refinement of the particle size distribution (PSD) was explored using hydrodynamic chromatography (HDC). An HDC method was developed and refined to separate large and small particles into separate fractions and reduce the overall PSD. Differences in chromatographic performance for columns packed after size refinement were observed and will be described.

Porous monoliths have been prepared inside pretreated 100 [micro]m I.D. fused silica capillaries using simultaneous sol-gel transition and polymerization of 3-(methacryloyloxy)propyltrimethoxysilane (MPTMS) in the presence of thermal initiator azobisisobutyronitrile. The MPTMS monomer used as the starting material contains inorganic as well as organic functionality and therefore preparation of hybrid monoliths is possible under specific reaction conditions. Initial experiments included analysis of bulk material by Fourier transform infrared spectroscopy (FT-IR). Acquired spectra aided in structure characterization by providing information about available functional groups. Optimization of preparation conditions towards enhanced column performance was considered including variations in the amount of porogen (70, 75, 80 vol% toluene), concentration of an aqueous hydrochloric acid catalyst (0.12 – 1 mol/L), polymerization time (3 - 96 hours), and temperature (60, 80, 100 [degree]C). Columns made using high HCl concentrations or low amount of toluene, and polymerized at high temperatures showed to be impermeable. The chromatographic performance of monolithic columns prepared under optimized conditions was assessed in reversed phase liquid chromatographic mode with respect to: retention factor, column efficiency, methylene and steric selectivity, effect of silanol groups, van Deemter plot, permeability, and pore size distribution. Columns with an efficiency of up to 117 000 plates/m for ethylbenzene has been achieved at a flow velocity of 0.32 mm/s.

Keywords: Chromatography, HPLC
Application Code: Nanotechnology
Methodology Code: Liquid Chromatography
Abstract Text
Nanodiamonds (NDs) have a variety of characteristics such as high thermal conductivity, excellent chemical, and mechanical stability that make them suitable materials for liquid chromatography (LC) or solid phase extraction (SPE). Of particular interest is their stability in a wide pH range, and the potential for multiple interactions with the ND surface. The goal of this work was to modify silica supports with NDs (4-5 nm) particulates for potential use in chromatography. Primary ND particles were obtained by a bead-assisted sonic disintegration method. The NDs were hydrogenated using a H2 atmosphere at elevated temperatures. Both, non-hydrogenated and hydrogenated ND primary particles were coupled to the surface of 1-µm silica particles. Modified silica particles were characterized by means of TEM, FTIR, and XPS. The obtained materials were packed into columns and tested under HPLC conditions. Selectivity of NDs packed column was directly investigated and compared to that of unmodified silica packed columns. This presentation will focus on the ND-silica preparation and the initial chromatographic evaluation of the ND-silica packed columns.

Keywords: HPLC Columns, Liquid Chromatography, Materials Science, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Liquid Chromatography
Lauryl acrylate based monolithic columns for nano-flow capillary HPLC were prepared in fused silica capillaries by thermal polymerization. The column efficiency of the resulting porous polymer monoliths (PPMs) was investigated. Past studies utilized capillary electrochromatography (CEC) to measure thermodynamic and kinetic characteristics of these PPMs. However, since electroosmotic flow is used to carry the mobile phase through the stationary phase in CEC, the experiments suffer from inaccurate volumetric measurements. Precise volumetric measurements of the mobile and stationary phases require knowledge of the flow rate, which HPLC provides. The properties that were measured include porosity, phase ratio, permeability, and optimal flow rates and sample concentration/injection volumes for these columns. Porosity is a measurement of the void spaces, therefore it is used to quantify the free space in monolithic columns. Total, external, and internal porosities were measured and the internal porosity of our PPMs was found to be significantly less than traditional monoliths found in the literature (Table 1). The phase ratio is the ratio of the volume of stationary phase to the volume of mobile phase in the column. The phase ratio was found to be 0.189 +/- 0.002. It was also verified to be independent of temperature. Permeability and efficiency were measured over a range of flow rates, from 100-1000 nL/min. This study presents the characteristics of the lauryl acrylate PPM and compares them to traditional and other monolithic stationary phases. This work is supported by the Petroleum Research Fund of the American Chemical Society and the National Science Foundation.

### Keywords
- Capillary LC
- Characterization
- HPLC Columns
- Separation Sciences

### Application Code
- General Interest

### Methodology Code
- Liquid Chromatography
### Session Title
LC: Column Technology

### Abstract Title
Analyte Diffusion Behavior on a Lauryl Acrylate Porous Polymer Monolith Stationary Phase

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### Abstract Text
Organic porous polymer monolith (PPM) stationary phases perform well in comparison to silica based monoliths and traditional particulate based phases. However, they have been incompletely characterized. By better understanding the interactions between analyte, stationary, and mobile phase in PPM systems, we not only improve our understanding of these new phases, but through comparison, we can be also improve our understanding of more familiar phases. The relationship between diffusion and retention of analytes on a lauryl acrylate PPM stationary phase was investigated. The behaviors of a homologous alkyl carbon series were probed using capillary electrophromatography and the peak parking method. The rate of axial diffusion within the stationary phase (D_{eff}), was determined at multiple peak parking times ranging from 0-75 minutes. These experiments were done in columns where the PPM had been polymerized in situ. Experiments were repeated in capillaries filled with only mobile phase to determine the diffusion coefficient of analytes, (D_m) in the mobile phase. One model of the relationship between retention and diffusion is described by the Knox equation (Knox and Scott, J. Chromatogra. 282, 1983, 297), D_{eff} = (\gamma_m D_m + k\gamma_s D_s)/(1 + k). Using this model, we have found that this PPM system follows Knox behavior, unlike traditional stationary phases that show deviation. These results, and explanations as to why these materials behave differently, will be presented. This work is supported by the National Science Foundation, the American Chemical Society Project SEED and Petroleum Research Fund, the Welch Foundation and the Howard Hughes Medical Institute.

### Keywords:
- Capillary Electrophoresis
- Characterization
- HPLC
- Separation Sciences

### Application Code:
General Interest

### Methodology Code:
Separation Sciences
We developed a novel open-tubular capillary which was coated with C60-fullerene by a covalent bonding via a photo/thermal active agent. We utilized perfluorophenyl azide (PFPA) as an active agent, which can be used for the “photo click” coupling of the carbon materials. The inner wall of a fused silica capillary was treated with silane conjugated PFPA, and then C60-fullerene was chemically modified by a photoreaction or a thermal reaction. As evaluations of the capillaries by liquid chromatography, the separation characteristics of polycyclic aromatic hydrocarbons (PAHs) were confirmed in both capillaries. With comparison of the separation selectivity to a commonly used C18 column, the prepared capillaries showed the specific separation ability based on the pi-pi stacking by C60-fullerene. The capillary prepared by the thermal reaction provided the base line separation of PAHs including phenanthrene, triphenylene, and benz[a]pyrene within 3 min at 18.8 cm capillary length.
This work presents the synthesis and physical characterization of 1.1 micron superficially porous particles with a thin porous layer and wide pores suitable for biological separations. Such particle architecture has become increasingly popular in recent years for the analysis of biological molecules. Commercially available superficially porous particles, however, are not as small as those presented here. This work also details the chromatographic performance of these particles when packed into capillary columns. The columns are first characterized by small molecule separations to determine their baseline performance. Then, these columns are characterized with larger biological molecules such as protein digests to evaluate their efficacy for biological separations.

Keywords: Capillary LC, HPLC, HPLC Columns, Liquid Chromatography
Application Code: Genomics, Proteomics and Other 'Omsics
Methodology Code: Liquid Chromatography
Higher speed and resolution in RPLC has been shown to be achievable for intact protein separations with silica colloidal crystals in capillaries using commercial nano-UHPLC instrumentation. Stainless steel columns are more robust than capillaries and are more widely used as an analytical tool for protein separations. This work examines the potential of using silica colloidal crystals of nonporous 470 nm silica particles packed in stainless steel columns, which are used with a commercial UHPLC instrument and UV detection. Preliminary results show that the peak width of ubiquitin is narrower than that for a leading commercial RPLC column. Both columns use a C4 bonded phase.

This work is supported by grant NIH R01 GM1011464

Keywords: HPLC, Liquid Chromatography, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Recently, we have discovered a new member of the macrocyclic chiral selector family called cyclofructan. Cyclofructans are cyclic oligosaccharides consisting of a crown ether core with pendent fructofuranose units spiro-annealed around its exterior. In their native forms, cyclofructans show little enantioselectivity when used as chiral selectors. However, when cyclofructans are derivatized and bonded to silica, they make exceptional chiral stationary phases for HPLC. Specifically, aliphatic (i.e. isopropyl) derivatized cyclofructan 6 exhibits tremendous enantioselectivity toward chiral primary amines primarily in the polar organic mode. Aromatic derivatized cyclofructans (i.e. dimethylphenyl and naphthylethyl) prove to be broadly selective for a wide range of types of racemates and typically perform optimally in the normal phase. In this study, the chiral recognition capabilities of three cyclofructan based chiral selectors, namely LARIHC CF6-P, LARIHC CF6-RN, and LARIHC CF7-DMP, were evaluated with supercritical and subcritical fluid mobile phases. The usage of carbon dioxide as a mobile phase component to replace either heptane (for transferring normal phase separations) or acetonitrile (for transferring polar organic separations) is evaluated. Using carbon dioxide as a replacement solvent allows for faster separations as well as huge operating cost savings. Direct comparisons between SFC and HPLC using the cyclofructan based chiral stationary phases will be made. Technical aspects of HPLC to SFC method transfer will be discussed in terms of flow rate, temperature, and mobile phase additives. Lastly, the applicability of semi-preparative SFC chiral separation on the LARIHC columns will be presented.

Keywords: Chiral Separations, Chromatography, Liquid Chromatography, SFC
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
### Abstract Text

This work describes the use of a reverse phase liquid chromatography (RPLC) column for size exclusion chromatography (SEC) of polysaccharides. The unique capability of the SEC-embedded RPLC is demonstrated in the separation of polysaccharides from other components in a glycol-conjugate vaccine or complex media of fermentation broth. The technique has also been applied to the separation of polysaccharides of different size. The effect of Chromatographic parameters including type of packing material (CN, C8 and C18), concentration of organic solvent and concentration of buffer on the separation was investigated. Theoretical modeling of the separation process is provided. The void volume and retention factor in SEC-embedded RPLC is re-defined to be suitable for both macromolecule and small molecule.

### Keywords:
- Bioanalytical, HPLC, HPLC Columns
- Pharmaceutical

### Gray Code:
- Liquid Chromatography
Capillaries packed with a silica colloidal crystal of 470nm nonporous particles have recently been shown to offer significant improvements in the peak width, resolution, and speed of protein analysis using a commercial nanoUHPLC. This is attributed to the reduction of the A and C term contributions of the Van Deemter equation as well as the presence of slip flow through the capillaries. While protein separations using this technology have shown vast improvements, small molecules had yet to be studied. As the silica colloidal crystal allows for an approximately diffusion limited separation, higher plate numbers are predicted for small molecules despite high diffusion coefficients. We have evaluated small molecule separations in both silica colloidal crystal packed capillaries and stainless steel columns with the goal of increasing resolution for pharmaceuticals.

This work is supported by NIH R01 GM101464

1. Rogers, B. J.; Birdsall, R. E.; Wu, Z.; Wirth, M. J., RPLC of Intact Proteins Using Sub-0.5 μm Particles and Commercial Instrumentation. Analytical Chemistry 2013, 85 (14), 6820-6825.

Keywords: HPLC, HPLC Columns, Liquid Chromatography
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Monoclonal antibodies and related products are the fastest growing class of therapeutic proteins. Ion exchange chromatography (IEC) offers a high throughput method for the characterization of charge heterogeneity of antibodies. The high molecular mass and large stokes radius of antibodies increase the mass transfer term, and consequently the theoretical plate height, thus decreasing column performance. Our approach to this problem is through the use of sub-2um nonporous silica particles to decrease the resistance to mass transfer term and increase column performance. The stationary phase was synthesized by grafting monomers with ion exchange groups onto the surface of nonporous silica particles by atom transfer radical polymerization. The resulting dense layer of flexible polyelectrolyte chains offer the selectivity needed in characterization of charge variants in therapeutic proteins while the solid silica core decreases the diffusion distance into and out of the stationary phase resulting in better column performance. Column performance was evaluated using ovalbumin as well as a therapeutic monoclonal antibody. Better resolution and selectivity were observed for both samples compared to a leading commercial ion exchange column.
Commercially available columns packed with core shell particles 1.3 µm in diameter have been shown to provide isocratic performance of nearly 500,000 plates/meter. The unique combination of ultra-high resolving power at high linear velocity shown by these sorbents can be used to increase resolving power while also reducing analysis time. In this presentation, the performance characteristics of Kinetex 1.3 µm core-shell sorbents when used for high-speed analyses requiring high resolving power will be explored in both gradient and isocratic elution modes. Relevant and challenging separations will be used to demonstrate the strengths and limitations of these unique materials. The effects of frictional heating at ambient and elevated column temperatures as well as approaches for reducing the impact of extra column variance in commercially available UHPLC instrumentation will also be discussed.

Keywords: High Throughput Chemical Analysis, HPLC Columns, Lipids, Peptides
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Characterization of Fullerene-Modified Silica as a Complement to Existing Alkyl Bonded and Graphite-Like Phases for Liquid Chromatography

Shortly after the discovery of fullerenes in the 1980s a few groups demonstrated their attachment to porous silica and polymer beads designed for use in liquid chromatography. At this time it was demonstrated that these fullerene-modified silicas (FMS) exhibited reversed-phase properties, and useful selectivity for highly aromatic isomers such as polyaromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). Since then, however, very little has been done in the way of characterizing other aspects of these materials. We have taken a keen interest in FMS with the idea that they may well complement the large number of existing alkyl (e.g., C18) and aromatic (e.g., phenyl) bonded silicas and graphite-like phases (e.g. porous graphitic carbon) as we continue the search for highly complementary phases for use in two-dimensional liquid chromatography (2DLC). In this presentation we will discuss the results of our initial attempts to characterize both the kinetic performance (i.e., with van Deemter study data) and chromatographic selectivity using both neutral and ionizable test solutes. We find that FMS not only exhibits useful isomer selectivity, as expected based on previous work, but also is quite different from both graphite-like phases and alkyl and aromatic bonded phases, as measured by linear retention models such as the Hydrophobic Subtraction Model. In our experience FMS is not an ideal stationary phase, but it has attributes that very nicely complement existing phases, especially where graphite-like phases have been disappointing historically (e.g., irreversible adsorption and terrible peak shapes for some compound classes). Much more investigation of FMS is clearly needed, however in our view the material has tremendous potential in 2DLC as a complement to existing phase chemistries.

Keywords: Food Science, Liquid Chromatography, Liquid Chromatography/Mass Spectroscopy, Separation Science
Metal nanoparticles (NPs) have interesting size-dependent optical, electronic, chemical, and magnetic properties, which are used for many applications, (e.g. catalysis, sensing and plasmonics). In catalysis, NP size is crucial. Smaller-sized NPs are the most effective and are attached to a solid substrate for certain applications. The stability of metal NPs against oxidation is a critical for these applications. We recently showed by voltammetry that the oxidation potential of Ag and Au NPs decreases as the NP size decreases. The decrease is about 200 mV from bulk to 4 nm average diameter for Au NPs electrochemically-synthesized. Here, we describe the direct voltammetric measurement of the oxidation potential (Ep) of chemically-synthesized Au NPs as a function of size. The chemically-synthesized Au NPs are attached electrostatically to indium tin oxide-coated glass (glass/ITO) electrodes functionalized with an amine-terminated silane linker. The oxidation potential of the chemically-synthesized Au NPs show less dependence on size and the Ep is more negative compared to the electrochemically-synthesized Au NPs for sizes >10 nm. We believe this difference is due to added stability gained by direct interaction of the electrochemically-synthesized Au NPs with the glass/ITO electrode. The electrostatic interaction of chemically-synthesized Au NPs with the organic linker is unstable. We also describe the influence of the electrode material on the oxidation peak potential (Ep) of 9 nm diameter citrate-capped Ag NPs. The Ag NPs were attached to Pt, Au, glassy carbon (GC), and glass/ITO electrodes electrostatically through short amine-terminated organic linkers. The Ep for Ag NP oxidation on the various electrodes followed the order of Au (Ep = 384 mV) > Pt (Ep = 372 mV) > GC (Ep = 351 mV) > glass ITO (Ep = 339 mV). Our results show that the electrode material affect Ag NP oxidation even if the Ag is attached through a linker and not directly interacting with the substrate.
Improvements in nanoscale fabrication techniques make continually smaller dimensions and higher degrees of molecular confinement easier to access. Translational diffusion in a restricted space results in an increased frequency of wall collisions, $[\omega_W]$, and the corresponding cumulative effect on surface adsorption events can no longer be discounted when describing molecular motion and designing lab-on-a-chip devices.

Diffusion in two principle systems is studied by Fluorescence Correlation Spectroscopy (FCS). Optically-accessible, horizontally-aligned nanochannels of nanometric height are ideal structures to study confined macromolecular transport in situ. These structures are directly applicable to lab-on-a-chip devices, but high degrees of confinement, characterized by the ratio of the channel height to molecular diameter, are currently difficult to achieve for small-to-midsize macromolecules. Using focused ion beam (FIB) milled zero-mode waveguides (ZMWs) enables smaller dimensions to be interrogated, corresponding to increased confinement. As they can readily be fabricated with both width and height below 100 nm, they can be correlated directly to the cross-sectional slices of nanopores and nanochannels.

Coupled to FCS, these two systems are platforms for single-molecule studies. Varying the degree of confinement and relating it to subsequent changes in molecular motion adds valuable insights into our understanding of transport at the nanoscale. Of particular significance are the subdiffusive tendencies of large, flexible molecules compared to small molecules, which show little to no deviation from bulk behavior. This work was supported by Department of Energy DE FG02 07ER15851.

**Abstract Text**

**Keywords:** Fluorescence, Lab-on-a-Chip/Microfluidics, Nanotechnology, Spectroscopy

**Application Code:** Nanotechnology

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Fluorescence Correlation Spectroscopy in Nanofluidic Channels: Effects of Confinement and Macromolecular Crowding on Molecular Transport

It is important understand the basic physics of transport in nanochannels, down to the level of the single molecule, in order to optimize the performance of nanoscale systems being developed for chemical analysis, sensing and diagnostics. The objective of this work is to develop and apply experimental measurements to understand various factors that will influence molecular transport under confinement and crowding in horizontal nanochannels. Fluorescence correlation spectroscopy (FCS), a highly sensitive technique to study molecular diffusion and chemical interactions and has been used to probe translational diffusion, active translocational transport and anomalous sub-diffusion in crowded nanochannels. The nanochannels are fabricated by using reactive ion etching (RIE) and photolithography on a silicon nitride layer. These horizontal nanochannels of varying heights (50-500nm) are used to quantify the effects of geometric confinement. Dextran of different molecular weights (4, 40 and 100 KDa) have been used to study the effect of excluded volume and increased viscosity on the molecular transport of macromolecules in the nanochannel.

Keywords: Fluorescence, Lab-on-a-Chip/Microfluidics, Nanotechnology, Spectroscopy
Application Code: Nanotechnology
Methodology Code: Microfluidics/Lab-on-a-Chip
Recently, there has been growing interest in development of the direct alcohol fuel cells as alternative technology to hydrogen based electrochemical energy systems. For example, ethanol (biofuel) can be ideally oxidized to carbon dioxide thus delivering twelve electrons. But realistically the reaction is rather slow at ambient conditions. Obviously, there is a need to develop novel electrocatalytic materials.

The commonly considered Pt anodes are readily poisoned by the strongly adsorbed intermediates, namely by CO-type species, requiring fairly high overpotentials for their removal. To enhance activity of Pt catalysts towards alcohols oxidation, additional metals including ruthenium, tin, molybdenum, tungsten or rhodium are usually introduced as the alloying component. Recently it has been demonstrated that catalytic activity of platinum-based nanoparticles towards electrooxidation of ethanol has been significantly enhanced through interfacial modification with ultra-thin monolayer-type films of metal oxo species of molybdenum or tungsten.

We pursue a concept of utilization of metal oxide matrices (Zr(IV), Ce(IV)) for supporting and activating noble metal nanoparticles (e.g. Pt, Pt-Ru) during electrooxidation of alcohols and formic acid. Remarkable increases of electrocatalytic currents measured under voltammetric and chronoamperometric conditions have been observed. The most likely explanation takes into account improvement of overall conductivity at the electrocatalytic interface (formed by metal oxide support), as well as possibility of specific Pt-metal oxide electronic interactions and existence of active hydroxyl groups on transition metal oxo species in the vicinity of catalytic Pt sites.

This work was supported by National Science Center (NCN), Poland under Maestro project.
Metal nanoparticles of various size, shape and composition are the catalysts of choice for a wide range of applications in energy technology. Typically, measurements made from such catalysts are the averaged responses from nanoparticle ensembles immobilized on a macro-scale electrode and can depend on the number of particles present and their spatial organization. Understanding the effect that size and shape have on the catalytic activity of individual nanoparticles is fundamentally important and remains a significant challenge.

Our goal is to measure the specific electrocatalytic activities of individual metal nanoparticles of various sizes, shapes and composition by immobilizing them on nanoelectrodes. These nanoelectrodes have been extensively developed in our lab for this purpose and are specifically designed to facilitate Electron Microscopy (EM) imaging of the attached particles. EM images of the nanoparticles allow the direct comparison of the electrocatalytic data to the individual nanoparticle attached to the electrode. We use the technique of Fast Scan Cyclic Voltammetry (FSCV), which allows real time monitoring of current step increases due to single nanoparticle collisions and also obtain multiple CVs of individual single nanoparticles as they land on the electrode. The nanoparticles have been tested for their activities towards reactions relevant to fuel cell operation, such as the hydrazine oxidation reaction. In our preliminary results we have found that many platinum nanoparticles with comparable diameters can display very different kinetic activities which may be related to the nanoparticle crystallinity and surface ligands present.
A novel catalyst free carbon nanotube (CNT) electrode was fabricated by a Carbo Thermo Carbide Conversion (CTCC) method. The morphology and nanostructure of the CNT electrodes were characterized by transmission electron microscopy (TEM) and Raman spectroscopy. Electrochemical characterization using potassium ferricyanide, ferroin and catechol shows these CNTs have higher current capacity, faster electron transfer rate and larger microscopic surface area than glassy carbon (GC) electrode. The potential applications of the CNTs were further studied in the detection of rare earth metal ion Eu³⁺ and neurotransmitter Dopamine. Good sensitivity and selectivity were obtained for both Eu³⁺ and Dopamine.
Electron transfer (ET) mechanisms and kinetics in dry, solid-state films of mixed-valent states of small (< 2 nm), monodisperse Au monolayer protected clusters (MPCs) will be discussed. Synthetic advances improving monodispersity have given impetus to understanding the core-size dependent ET properties of Au MPCs. It is also worthwhile to characterize the ET behavior of these atomically precise nanoclusters at low temperatures to open the door for future applications. The ET behavior demonstrated by films of Au MPCs were found to exhibit a wide range depending on core size and temperature. We show that ET rates are charge-state and core-size dependent for small Au MPCs at both high and low temperatures. At reduced temperatures, the thermally activated ET process dissipates, revealing non-Arrhenius behavior, as ET rates become independent of temperature. Recent ET studies on other nanoparticle systems will also be discussed.
Due to the potential for human carcinogenesis, the control of genotoxic impurities (GTIs) at trace levels is required within pharmaceutical products. Determination of GTIs at ppm level requires very sensitive analytical methodology which may create challenges for method development. The challenge is amplified when the GTI analyte does not possess a UV chromophore and the selectivity is problematic due to the fact that the GTI analyte is at low level in the presence of relatively large amounts of drug substance and excipients. An established mutagen, 4-chlorobutanol is formed as a result of the reaction of HCl with THF. In order to achieve the desired control limit of 1 ppm, a direct injection gas chromatography with flame ionization detection (GC-FID) method was developed for analysis of 4-chlorobutanol in the drug substance. A high bias in the accuracy results was found, and after an extensive investigation this was determined to be due to use of a specific liner and the diluent selected. The method development and investigation will be presented. Due to a substantial amount of 4-chlorobutanol being present in the input drug substance, it was essential to also develop and validate quantitative methods for this GTI in the drug products. GC-headspace was initially tried to minimize potential matrix interferences, however the reverse reaction of 4-chlorobutanol to THF was observed that prevented the progression of this technique. Instead, similar method conditions were utilized as for the drug substance, with additional modifications such as the use of guard column and the sample purification procedure on order to overcome the effects of the matrices in three different formulations. The methods were successfully validated and used to support batch analysis for quality control. The approaches used to analyze the product matrices will be presented.

Keywords: Gas Chromatography/Mass Spectrometry, GC Detectors, Method Development, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Gas Chromatography
Pharmaceutical: GC, LC/MS, Raman Spectrometry, Capillary Electrophoresis and Separation Sciences

Electrochemiluminescent Microchip and LC-MS/MS for Organ-Specific Reactive Metabolite Profiling

Reactive metabolites and their adducts with DNA are important genotoxicity biomarkers for drugs and environmental chemicals. In addition, elucidation of organ specific genotoxic chemistry promises to provide more informative profiles of chemical effects on humans. A reliable, high-throughput platform providing organ-specific molecular information that augments predictions from toxicity bioassays would be an attractive addition to drug and chemical development toolkits.

In this paper we describe a high-throughput fluidic chip capable of screening reactive metabolites generated by a representative set of human organ enzymes. The chip features printed 1 [micro]L micro-wells fabricated to contain films of DNA, electrochemiluminescent (ECL) polymer [Ru(bpy)2(PVP)10](ClO4) {(PVP = poly(4-vinylpyridine)} and enzymes from liver, lung, kidney and intestine in separate spots. The device first runs metabolic reactions specific to each organ under constant reactant feed, then generates ECL light related to the extent of DNA damage caused by formation of DNA-metabolite adducts. Identities and formation rates of DNA adducts were confirmed by capillary liquid chromatography-tandem mass spectrometry (CapLC-MS/MS), using similar films of DNA/enzyme on magnetic bead biocolloid reactors in 96-well plates, followed by DNA hydrolysis and CapLC-MS/MS analysis.

Keywords: Liquid Chromatography/Mass Spectroscopy, Luminescence, Metabolomics, Metabonomics

Application Code: Pharmaceutical

Methodology Code: Liquid Chromatography/Mass Spectrometry
Well designed and executed excipient compatibility studies are the essential first step in pharmaceutical development of successful formulation and dosage forms. When incompatibilities between an API and excipients do occur, degradation products are to be identified to understand the mechanisms of formation and to guide further development activities such as formulation designs, package configurations, and long-term stability studies. In some cases, it may be necessary to proactively qualify some degradation products in order to achieve greater flexibility in the development of a program.

This presentation describes the analytical development effort in conducting excipient compatibility studies for formulation development of a small molecule active compound. With samples stored at 60 °C and 50°C/75%RH in open and closed containers, the API was found to have significant chemical compatibility issues (up to 40% after 2 weeks) with some commonly used excipients such as mannitol and stearic acid. This is despite the acceptable stability observed for the API alone. Different degradation profiles were observed under elevated temperature with or without humidity control. Identification of the major degradation products by HPLC/HRMS revealed the complicated degradation mechanism involving primarily hydrolysis and oxidation. The final selected formulation was assessed again using ASAP program to gain additional insight on the long-term stability of the formulation and shelf-life of the drug product. The excipient compatibility study results clearly demonstrated the need for moisture protection of the final solid dosage form.

Keywords: HPLC, Identification, Mass Spectrometry, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Pharmaceutical: GC, LC/MS, Raman Spectrometry, Capillary Electrophoresis and Separation Sciences

The New Reality Show - Can HPLC Keep Up With Fast LCMS?

Not so long ago, HPLC was slow and LCMS detectors were able to keep up. The need for sub 1 minute HPLC analysis has pushed the development of LCMS instruments to faster operation. Mass spectrometers can now detect and quantitate HPLC peaks that are only seconds wide. It is becoming common to analyze hundreds of compounds in under a minute. But where do we go from here? Peak widths are now down in the sub-second range. The typical solution has been to run these detectors in a SIM or MRM mode and avoid polarity switching. While that may be suitable when you know what you want to analyze, it doesn’t always work when you might have an unexpected contaminant show up or if you want to analyze a drug and all its metabolites at incredibly low levels. And even if you have the correct analyte, it isn’t always obvious how it will ionize. Until now, HPLC developments have moved faster than LC/MS has been able to keep up. Now, with new approaches in the design and operation of triple quadrupole mass analyzers, it is possible to perform LCMS analysis so fast, even with dual polarity measurements, that the fastest UHPLC is now considered slow. Results will be shown for HPLC applications with exceptionally short run times with high peak densities and at high sensitivity.

Keywords: HPLC, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
This talk focuses on the significant benefits of transmission Raman spectroscopy (TRS) as an alternative content uniformity release method to HPLC for tablet and capsule manufacture. TRS enables rapid method development, good accuracy, measurement times of seconds per tablet, requires no sample preparation (e.g., of coated tablets and capsules), no consumable items and routine testing can be performed without requiring skilled analysts. Content Uniformity analysis for tablets and capsules is required by regulatory bodies for release of a pharmaceutical production batch to ensure the correct patient dose of their active pharmaceutical ingredients (APIs). Individual tablets or capsules must be analysed, typically by an HPLC method, which is resource-intensive, slow, expensive and requires ongoing consumable commitments. Content uniformity analysis by a spectroscopic technique is attractive because of a significant reduction in ongoing testing resource. However, the most common method – near infra-red spectroscopy – has issues of complicated method development and maintenance, difficulties with samples containing moisture or variances in particle scattering or coatings and of samples more than ca. 4mm thick.

This talk discusses the use and benefits of TRS using a variety of practical examples and with reference to the development of methods according to regulatory best practices. TRS is a viable alternate release method that can be implemented for a wide variety of products and dosage forms.
Heparin is a complex biopolymer used as an anticoagulant. This research uses capillary electrophoresis to investigate heparin contamination; specifically, how contamination of heparin will affect heparin’s ability to act as a chiral selector. Initial work has focused on optimization of the chiral separation of the enantiomers of pheniramine and chloroquine using heparin. Optimized separation conditions were used to examine heparin contaminated at various levels. Separation characteristics, such as resolution and electrophoretic mobility were measured for each of the contaminated samples. Results indicate that this optimized method shows promise in determining heparin contamination.
Supercritical Fluid Chromatography (SFC) is a useful tool for analytical and large scale separations. SFC is most often used with carbon dioxide as a mobile phase and an organic modifier such as some type of organic alcohol. It has some significant advantages over standard HPLC methods such as less pressure drop across the columns, faster column equilibration, faster method development, higher efficiency separations and significantly less generation of hazardous waste. Some of the main advantages for preparatory chromatography include solvent waste reduction, facilitated product recovery, lower solvent cost and the possibility for recycling. One major advantage of using Supercritical Fluid Chromatography for large scale separations is the ability to recover the CO2 and recycle it concurrently with executing the process. Contained in this study we have demonstrated CO2 recycling with multi-compound mixtures and Chiral compounds that yield two or more peaks for overnight periods. In using multi-compound and chiral mixtures a more accurate picture of the capability of the Recycler being used is portrayed. The body of work will include Retention Time RSD% and effects of slight co-solvent/modifier changes with regards to retention time. Recycle efficiency for the methods and conditions used in the multi-component tests will also be discussed. Our data will show the high recycling efficiency (RE) with the designed system and excellent chromatographic performance for the separations. Recovery data will be included as a validation of the results of the testing.

Keywords: Chiral Separations, Chromatography, Prep Chromatography, SFC
Application Code: Pharmaceutical
Methodology Code: Separation Sciences
This talk focuses on a new technology with the unique ability to identify a wide range of pharmaceutical raw materials without opening their original packaging, even if the packaging is not transparent. Samples can be released into production quickly from the loading dock, without sampling booths or long delays in quarantine. Because the container is not compromised there are also benefits in the manufacture of sterile products.

Raman spectroscopy is being increasingly used for raw materials identification because it is quick, chemically selective and easy to use. However, conventional Raman typically works through transparent colourless containers such as thin plastic bags. A new technique, spatially offset Raman spectroscopy (SORS), enables Raman analysis of the contents of paper and plastic sacks, tubs, bottles and other thick and/or opaque containers. Examples of relevant incoming goods that work with SORS include lactose and dextrose in multi-layer paper sacks, APIs in plastic tubs, liquid excipients in amber glass bottles and HPMC in plastic sacks. This talk describes how the technique works, what samples it can be applied to and how it is being used in pharmaceutical companies.

**Keywords:** Identification, Infrared and Raman, Pharmaceutical, Quality Control

**Application Code:** Pharmaceutical

**Methodology Code:** Portable Instruments
Safety, health, and environmental concerns of conventional solvents such as hexane and related hydrocarbons stimulate the use of green solvents in the extraction processes. Green solvents have been characterized for their low miscibility, high boiling point, and easy biodegradability to nontoxic products under environmental conditions, which makes them safer and less risky to human health and environment. Oils were extracted from soybeans using green solvents such as d-limonene, 2-methyltetrahydrofuran, cyclopentyl methyl ether, ethyl lactate, tert-butyl methyl ether, [alpha]–Pinene, supercritical carbon dioxide and deep eutectic solvents. The extractions were carried out using accelerated solvent extraction and supercritical fluid extraction. We studied the extraction kinetics with these green solvents and compared the experimental data to the hot-ball model. The performances of these green solvents compared to the conventional hazardous solvents in the extraction of soybean oil were validated.
Sampling and Sample Preparation for the Food Sciences

Extraction of Caffeine from Tea and Water Using QuEChERS with Gas Chromatography/Mass Spectrometry Detection

Caffeine is a model system applied to many areas of chemistry including organic, environmental, and pharmaceutical analysis. The use of such a model system is of interest because it is ideal for the investigation of fundamental chemistry parameters including partition coefficients and extraction conditions and their affect on extraction kinetics and recoveries for the model analyte. While caffeine is a model analyte, tea has qualities of both food and plants, providing a model complex matrix for the investigation of matrix interferences. This complex matrix provides a system in which the sample preparation method QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) can be applied. In this work, extraction conditions of the QuEChERS method were varied and partition coefficients as well as kinetic effects were studied. The extraction steps of QuEChERS includes both a liquid-liquid microextraction and a dispersive solid phase extraction (d-SPE) clean-up step which provides a clean sample for GC-MS analysis by removing matrix interferences as well as limiting solvent use. Throughout the literature there are many application studies involving QuEChERS; however, investigation of the fundamental aspects of this method has been limited. Acetonitrile, ethyl acetate, and ethanol/water mixtures were investigated as solvent systems for this study, where the optimum extraction conditions, partition coefficients, and the kinetics of the extraction of caffeine for each system were determined. The extraction conditions and kinetics will be discussed along with percent recoveries and the depletion study used to calculate extraction ratios for the determination of partition coefficients.

Keywords: Food Science, GC-MS, Sample Preparation, Separation Sciences
Application Code: Food Science
Methodology Code: Sampling and Sample Preparation
Pesticides are used on agricultural commodities such as table grapes and wine grapes to protect against insects, fungi, mold and other agents that may affect crop yield, cosmetic appearance, and flavor properties. Wine being a major agricultural commodity, it is important to know the types and quantities of these pesticides that are present in the product as these affect the overall purity, quality and safety to consumers. As a result, many analytical methodologies have been created to monitor these compounds in this food matrix. Given the amount of samples that must be tested in any given year, it is necessary to develop fast automated SPE methodology for the extraction of these compounds from wine, and also post extraction treatment of extracts prior to GCMS analysis.

We have developed an automated SPE method for the extraction of 20 organochlorine pesticides. The method uses the SmartPrep Automated Cartridge Extractor System using Amino Propyl and HLB cartridges as well as DryDisk membrane technology for drying extracts. We will show the efficiency of the extraction while demonstrating good recoveries of these 20 organochlorine pesticides. This method will demonstrate an improvement to existing extraction methodology, and also model an extraction scheme that can be used for other organochlorine pesticides as well as Organophosphorous pesticides and Antifungal compounds.

**Keywords:** Food Science, Sample Handling/Automation, Sample Preparation, Solid Phase Extraction

**Application Code:** Food Science

**Methodology Code:** Sampling and Sample Preparation
This study shows results obtained from a systematic investigation of the adsorptive behavior of solid porous sorbents, as solid phase microextraction coatings, in extractions from a complex matrix. The relevance and advantages of the current study as applied to plant metabolomics and generally to the analysis of complex food matrices have also been mentioned. Notwithstanding the advantages of extracting compounds with varying degree of molecular weights and polarities, the issue of competitive adsorption/displacement of compounds often affects the overall method extraction efficiency. In this regard, the extent of competitive adsorption was evaluated by comparing headspace (HS) and direct immersion (DI) sampling modes, at different extraction times using commercially available solid sorbents. In addition, the recently introduced solid coatings overcoated with PDMS were also analyzed. Results showed that the extraction mode does have a significant influence on the displacement effect for the commercially available coatings. The PDMS-modified solid sorbent coatings not only demonstrated remarkable matrix compatibility, as mentioned previously, but also reduced the effect of inter-compound displacement, thus, making it a clear choice suitable for direct sampling in complex matrices. To complement the present investigation, a model food matrix was employed to simulate ex-vivo and in-vivo sampling conditions. Selected compounds of different functionalities were used as a model to represent metabolites occurring in plant-based substances. The results obtained further support the relevance of the direct immersion SPME as a means to avoid or limit the occurrence of inter-analyte displacement in complex matrices. The impact of these findings shows the potential of in-vivo SPME strategies for quantitative metabolomics studies of complex plant-based systems.

Keywords: Food Science, Metabolomics, Metabonomics, Sample Preparation
Application Code: Food Science
Methodology Code: Sampling and Sample Preparation
Competitive adsorption or displacement effect is a characteristic phenomenon observed in solid porous sorbents often when applied as an extraction phase in complex matrices such as food. This is due to the adsorptive extraction mechanism at surface active sites, which allows molecules to be physically trapped to the sorbent through weak intermolecular interactions. Thus, in complex matrices, molecules with higher affinity for the sorbent tend to replace lower affinity compounds. Notwithstanding, the introduction of solid porous coatings in solid phase microextraction (SPME) applications has facilitated the analysis of molecules varying molecular weights and polarities in various matrices, especially food.

Recent work, demonstrated the successful application of solid coatings in SPME for fruit metabolomics study. Although, general understanding of the displacement effect has been linked to analytes’ polarities in a given matrix, recent trends in the application of solid porous coatings in SPME make it valuable studying the phenomenon further. Therefore, in this study the role and extent of the functionalities of the compounds in a given matrix and their influence on the competitive adsorption process have been presented. The displacement effect was carried out using compounds having different functionalities. In order to maintain its relevance to food applications, a group of metabolites often found in food matrices were selected as model analytes. The results confirmed the influence of the compounds functionalities in the competitive adsorption process in addition to their polarities.
Analytical Pyrolysis is used to characterize non-volatile samples, like plastics, paper, paint, and textile, mostly in combination with GC/MS. The samples are thermally degraded in an inert atmosphere and the pyrolysis products are separated and identified. Qualitative information is obtained by identifying specific pyrolysis products or by comparing with reference substances. Quantitative information can be obtained by measuring the amount of pyrolysis products.

By better understanding of the pyrolysis process it is possible to maximize the information obtained. Secondary reactions that occur after pyrolysis can be reduced and the influence of sample size can be minimized. The fundamental influence of pyrolysis temperature and time on the pyrolysis products will be discussed, and a systematic method for choosing pyrolysis conditions is presented.

With an unknown substance the use of fractionated pyrolysis, when the same sample is pyrolyzed repeatedly at different temperatures, is especially well suited. Each pyrolysis is analyzed separately on the GC or GC/MS, and the sample is allowed to cool down to the chamber temperature between the GC runs. At the chamber temperature, typically 175 degrees C, the pyrolysis reactions stops. The methodology is demonstrated with examples.

**Keywords:** Analysis, Characterization, Polymers & Plastics, Pyrolysis

**Application Code:** Quality/QA/QC

**Methodology Code:** Sampling and Sample Preparation
The increasing importance of obtaining accurate and reliable information about the concentration of bioactive compounds in natural products on a timely fashion and with a lower environmental impact is prompting the development of new techniques and methods. Modern sample preparation techniques, such as ultrasound-assisted extraction, pressurized-liquid extraction (PLE), supercritical-fluid extraction (SFE) and solid phase extraction (SPE), can provide several advantages. These techniques are also suitable for automation and on-line coupling with chromatographic techniques [liquid chromatography (HPLC) and supercritical fluid chromatography (SFC)]. In this work we developed an integrated sample preparation and analysis system for the determination of bioactive compounds in natural products exploring these techniques. The system is capable of performing Ultrasound-assisted PLE (UAPLE) or Ultrasound-assisted SFE (UASFE), coupled on-line with the purification step (SPE) and analysis by HPLC or SFC. Any of these techniques can be performed individually or combined/ coupled on-line. Different combination of these sample preparation techniques (PLE, SFE with or without ultrasounds and SPE) and analysis (HPLC and SFC) is currently being explored as a new strategy to improve the whole processe and reduce the consumption of organic solvents for the analysis of bioactive compounds, including isoflavones from soybeans, polyphenols from coffee, tea and champignon and curcuminoids from curcuma longa rhizomes. Results indicate that, in most cases, the developed system is capable of producing better results than the conventional techniques but required a larger initial investment in equipment. Funding from FAPESP (Project 2013/04304-4) is acknowledged.

**Keywords:** HPLC, Natural Products, Sample Preparation, SFC

**Application Code:** Food Science

**Methodology Code:** Sampling and Sample Preparation
### Session Title
Electrochemistry: Methods and Applications

### Abstract Title
**The Use of Microelectrode Voltammetry to Determine n-octanol / Water Distribution Ratio of Electroactive Species**

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### Abstract Text
The present work shows the use of microelectrode voltammetry on the n-octanol/water determination of partition and distribution ratios for electroactive species as methylene blue, hydroquinone, ascorbic acid and acetaminophen. Generally, the determination of partition and distribution ratios is carried out by shake-flask method, and the most common method of measuring the equilibrium concentration of the solute on both phases is UV/vis spectroscopy. However, the use of this technique can lead to unreproducible results due to: (i) the adsorption of the solute on tubes and cuvettes; (ii) aggregation factors; (iii)solutes that increase the miscibility between the solvents, altering the refractive index of both phases; amongst others. Thus, the development of an alternative method for the partition or distribution ratio determination is desirable. The use of voltammetry with microelectrodes has some advantageous features compared to that carried out with conventional electrodes. Regarding the determination of the partition ratio, the main advantage is the possibility of performing voltammograms in media with low electrical conductivity due to the low intensity of current supplied by the microelectrodes, ie there is no need to add electrolyte to the aqueous solution for measurement. All the voltammograms were performed using an Autolab PGSTAT 128 N potentiostat employing a three-electrode electrochemical cell: carbon fiber or platinum microelectrode as working electrode, Ag/AgCl electrode as reference electrode and a platinum wire as auxiliary electrode. The methodology is based on obtaining two voltammograms in aqueous phase: one before and one after the partition. As the limiting current (I[\text{sub}L/\text{sub}]) of voltammograms are proportional to the concentration of electroactive species in solution, \( \log D \) values were calculated by the difference of the \( I[\text{sub}L/\text{sub}] \) before and after the partition divided by \( I[\text{sub}L/\text{sub}] \) before the partition. FAPESP

### Keywords
Electrochemistry, Microelectrode, Voltammetry

### Application Code
General Interest

### Methodology Code
Electrochemistry
Hydrogen peroxide is a bleaching agent used in industrial consumer products, therapeutic applications and water treatment. Hydrogen Peroxide is used as a disinfectant in water purification and waste water treatment. Therefore there is a need to monitor the various water samples for hydrogen peroxide. Hydrogen peroxide used for disinfection also decomposes in the presence of heat or light and therefore needs to be monitored carefully. Measurement of atmospheric hydrogen peroxide also requires analytical methods to monitor its fate. The presence of hydrogen peroxide in air degrades air quality. Beverage samples such as cola drinks are also known to contain hydrogen peroxide and needs to be monitored. Tooth pastes and whitening kits also contain hydrogen peroxide and needs to be analyzed.

In this presentation we show a new ion chromatography method using a CarboPac PA20 column and potassium hydroxide eluent and electrochemical detection (ED) to detect trace levels of hydrogen peroxide from various samples as outlined above. A reagent free ion chromatography (RFIC) system was used in this analysis and provided a simple setup to pursue this application. The method performance in terms of linearity, reproducibility and detection limit is presented here.

**Abstract Text**

Hydrogen peroxide is a bleaching agent used in industrial consumer products, therapeutic applications and water treatment. Hydrogen Peroxide is used as a disinfectant in water purification and waste water treatment. Therefore there is a need to monitor the various water samples for hydrogen peroxide. Hydrogen peroxide used for disinfection also decomposes in the presence of heat or light and therefore needs to be monitored carefully. Measurement of atmospheric hydrogen peroxide also requires analytical methods to monitor its fate. The presence of hydrogen peroxide in air degrades air quality. Beverage samples such as cola drinks are also known to contain hydrogen peroxide and needs to be monitored. Tooth pastes and whitening kits also contain hydrogen peroxide and needs to be analyzed.

In this presentation we show a new ion chromatography method using a CarboPac PA20 column and potassium hydroxide eluent and electrochemical detection (ED) to detect trace levels of hydrogen peroxide from various samples as outlined above. A reagent free ion chromatography (RFIC) system was used in this analysis and provided a simple setup to pursue this application. The method performance in terms of linearity, reproducibility and detection limit is presented here.

**Keywords:** Air, Electrochemistry, Ion Chromatography, Water

**Application Code:** Environmental

**Methodology Code:** Liquid Chromatography
Detection of Thiols by o-quinone Nanocomposite Modified Electrodes

In nature, thiols are widely distributed and prominent marker molecules in living cells. They are antioxidants which are involved in many oxidation-reduction reactions in many biological and chemical systems. In particular, thiols protect living cells against oxidative stress. Accurate determination of the changes in thiol levels provides critical results for diagnosis of disease states. Numerous chemical and instrumental techniques for the determination of thiols have been reported and many techniques suffer from difficulties such as stability and sensitivity. Therefore, it is a challenge to develop new biosensors to accurately detect and quantify thiols. Herein, new immobilization matrices, co-electrocatalysts and immobilization methods were evaluated using coenzyme pyrroloquinoline quinone (PQQ) as the primary electrocatalyst to develop new biosensors for accurately detect and quantify thiols. As revealed by preceding investigations o-quinone moiety of PQQ shows a reversible 2-electron, 2-proton oxidation/reduction reaction and it is known to catalyze reactions of the oxidation of thiols to disulfides. Therefore thiols can be determined at lower overpotentials by amperometric detection during the oxidation of the reduced form of PQQH2 to PQQ. Nanoparticles which incorporated into the polymer matrix, such as gold (Au NP) and single wall carbon nanotubes (SWNTs) exhibit novel electronic properties making them suitable for enhancing electrochemical sensitivity. During this investigation cysteine and homocysteine were detected quantitatively at micromolar level by incorporation of PQQ and SWNTs-COOH into a Au-PPy nanocomposite conducting polymer matrix on a glassy carbon electrode surface.

Keywords: Biosensors, Detection, Electrodes
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Quercetin (3, 3’, 4’, 5, 7-pentahydroxy-flavone) is part of a subclass of flavonoids called flavonols that has received considerable attention because of its wide range of potential health benefits which include antioxidant and anti-inflammatory properties. Quercetin protects the body against several degenerative diseases such as diabetes, arthritis and cancer. In this report, the levels of quercetin in 20 tropical fruits and vegetables were determined by cyclic voltammetry using glassy carbon as working electrode, Ag/AgCl as reference and platinum as auxiliary electrode in 0.1M phosphate buffer pH 7.4. The measurements were made in a potential range of -500 mV to 1200 mV using a scan rate of 100 mV/s. The peak current for the oxidation of quercetin in the samples was recorded at 366 mV. The concentrations of quercetin determined in the fruit and vegetable samples were in the range 4.77 mg/100 g (green pepper) - 28.72 mg/100 g (red apple). The report will serve as a guide in the selection of tropical fruits and vegetables with appreciable levels of quercetin.

Keywords: Bioanalytical, Biological Samples, Detection, Electrochemistry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Our recently developed electrochemical proximity assay (ECPA) is a highly sensitive and selective direct-readout method for quantitative analysis of proteins at femtomolar levels. Antibody-oligo or aptamer-based probes are employed, with square wave voltammetry (SWV) as the readout. Current density, proportional to protein concentration, originates from redox label molecules attached to signaling DNA strands, such as methylene blue (MB), ferrocene (FC), etc.

To optimize SWV frequency for MB in HEPES/NaClO4, we employed a DNA-loop model from our previous work and monitored current at frequencies from 10 - 150 Hz. 75 Hz with 90 mV pulse height gave 4-fold higher signal than 60 Hz and 50 mV (prior conditions) for MB-labeled DNA. By stirring 20-µL samples and using 7-bp signaling DNA, combined with the above parameters, the concentration sensitivity of antibody-based insulin ECPA was increased by 10.4-fold. The same method was extended to determine the optimal parameters for FC-labeled DNA, with different sets of DNA sequences to avoid interference. 90 Hz with 50 mV pulse height gave optimal signal for FC labels.

To conclude, there are about five redox labels which are commonly used, all being distinguished by their respective redox potentials (for example, MB = -210 mV, FC = 310 mV (vs Ag/AgCl)). Here, it is observed that redox labels are also distinguished by optimal SWV frequencies, which should allow multiplexing in two dimensions (potential and frequency, see Figure 1). This study should pave the way for effective multiplexed detection of proteins at femtomolar levels via direct readout using ECPA.

Keywords: Analysis, Bioanalytical, Biosensors, Electrochemistry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Selective Detection of Pyocyanin in Biological Samples Using Disposable Electrochemical Sensors

The use of electrochemical detectors to provide a rapid, sensitive diagnostic platform for biologically relevant molecules is well documented (i.e. glucose monitoring). Additionally certain virulence factors produced by bacteria can be measured electrochemically. Diagnosis of bacterial infections could be aided by the early detection of these factors in medically relevant fluids. Here we report the selective and sensitive detection of pyocyanin from the opportunistic pathogen Pseudomonas aeruginosa in whole blood, urine, sputum, and bronchial lavage solutions, as well as in growth media with unmodified, disposable screen-printed carbon working electrodes.

Square wave voltammetry (SWV) scans were performed of blood, urine, sputum, and bronchial lavages spiked with dilutions of pyocyanin to show the electrochemical detection of pyocyanin. Biological fluids spiked with the virulent P. aeruginosa strain PA14 were also scanned. We also screened, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermis, and Bacillus cereus via SWV for the presence of other electro-active molecules that would interfere with the detection of pyocyanin supernatants from cultures of P. aeruginosa.

The selective, linear detection of pyocyanin from the range of 1-100 µM was achieved in samples spiked with pyocyanin. No molecules other than pyocyanin (in P. aeruginosa cultures only) were detected in the potential range from -0.5 to 0 volts. Samples of P. aeruginosa spiked in biological fluids showed a pyocyanin response after one day of culturing at 37 degrees Celsius. The results indicate the potential to sensitively and selectively detect the presence of P. aeruginosa in relevant biological samples.

This work was supported by the U.S. National Science Foundation under Grant #1125535.
Cystine, an Essential Determinant of Protein Tertiary Structure, is Also a Target for Electrochemical Manipulation

The ability for thiol residues to undergo oxidation to disulfides is biochemically important affecting antioxidant activity (e.g., glutathione) and determining protein tertiary structure. The formation of cystine disulfide bridges from two cysteine residues not only influences protein confirmation but also renders it electrochemically active. Although peptides can be quantified by electrochemical oxidation of the disulfide bond, the commonly used working electrode, glassy carbon, suffers from two major issues – a limited oxidation potential and the persistent fouling of the electrode’s surface necessitating frequent cleaning procedures. The boron-doped diamond (BDD) working electrode, on the other hand, can be used at extreme oxidation potentials and, as its surface is inert, does not suffer from adsorption problems. The ability for HPLC with electrochemical detection to quantify a variety of disulfide containing peptides was evaluated and included measurement of glutathione disulfide, cyclic nonapeptides (vasopressin and oxytocin), an example of intra and inter chain disulfide cross-linking (insulin) and a cyclic, disulfide rich cyclotide (cycloviolacin O2).

Furthermore, the inclusion of a BDD working electrode prior to the electrospray source of a mass spectrometer can be used to manipulate protein/peptide structure prior to detection and includes breaking of the disulfide bond and cleavage of the amide backbone at tryptophan and tyrosine residues. The use of this technique for the above examples will also be discussed.

Keywords: Electrochemistry, HPLC Detection, Peptides, Protein
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
The flavoprotein Pyranose 2-oxidase (POx) [1] attracts interest as potential component in enzymatic biofuel cells (BFCs) [2]. POx catalyses the oxidation of aldopyranoses, preferred D-glucose, using molecular oxygen as e\(^{-}\) acceptor. Alternative e\(^{-}\) acceptors are benzoquinone, radicals and chelated metal ions [3]. In this study semi-rational protein design was used to find variants with decreased activity toward oxygen, in order to avoid subsequently production of H\(_2\)O\(_2\). Five mutants were identified using a screening assay in 96-well plates with ABTS and 2,6-Dichlorophenolindophenol (DCPIP), which reacts directly with the reduced FAD [4]. The POx mutants showed drastically decreased oxygen activity, 0.1 - 20 % compared to wild type POx resulting from increased \([i]K_M[/i]\)/\([i]k_cat[/i]\) values and decreased turnover numbers. When DCPIP and 1,4-benzoquinone (1,4-BQ) were used as e\(^{-}\) acceptors in the spectrophotometrical activity assay, three mutants showed up to four times higher catalytic efficiencies in comparison to the wild type; the affinity to DCPIP and 1,4 BQ was enhanced and accompanied by equal or increased \([i]k_cat[/i]\). As a result the dehydrogenase/oxidase ratios of the identified five POx mutants were 10 to 100-fold higher compared to the wild type POx. The next step is to immobilize the variants on graphite electrodes and to determine the energy output using flow injection analysis as it was already successfully performed in 2010 [1].

Financial support: BOKU Doc Programme, Austrian Science Fund (FWF), Doctoral Programme BioToP (FWF W1224) and individual project P22094

2 Spadiut, O, Brugger, D, Vasile, C, et al. 2010 Electroanal. 22, 813-820

Keywords: Biotechnology, Electrochemistry, Enzyme Assays, Genetic Engineering
Application Code: Other (Specify)
Methodology Code: UV/VIS
Abstract Text

Due to its ability to biodegrade in aqueous containing chloride environment, magnesium and its alloys have attracted significant interest in biomaterials research to develop biodegradable implants for bone repair. However, the effect of magnesium and its alloys on cells and surrounding tissues during in vitro corrosion studies needs to be studied extensively before making conclusions about biocompatibility. This need arises from the fact that cell death can also result from high pH or high osmolality, leading to osmotic shock. Therefore there is a need to develop an electrochemical sensor that can estimate osmolality from measured conductivity during in vitro corrosion studies. In this work, 0.9% sodium chloride solution, phosphate buffered saline (PBS), Hank’s balanced salt solution (HBSS) and Dulbecco’s minimum essential media (DMEM/F12) were used as model electrolyte solutions for in vitro corrosion studies of pure magnesium, to demonstrate sensors for measuring pH and osmolality under cell culture conditions. The surface of the corroded pure magnesium in each test solution was characterized by scanning electron microscopy (SEM), energy dispersive x-ray spectroscopy (EDX), and attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). Conductivity measurements taken in each test solution containing pure magnesium increased during a 48 hour immersion period at 37°C and could be used to estimate changes in osmolality.

Keywords: Electrochemistry, FTIR, Metals, Sensors

Application Code: Biomedical

Methodology Code: Electrochemistry
**Abstract Text**

Dopamine (DA) and norepinephrine (NE), the major catecholamines in the brain, have been implicated in a variety of physiological functions and behavioral responses and play important roles in diverse neurological and psychiatric disorders such as Parkinson’s disease, Alzheimer’s dementia, and addiction. Accumulating evidence suggests that NE regulates the activity of dopaminergic neurons of the ventral tegmental area (VTA) through noradrenergic receptors (alpha 1- and alpha 2-adrenergic receptors) presented in the VTA. In this study, we investigated the contribution of norepinephrine to activity of the VTA dopaminergic neurons of urethane anesthetized rats. The neurochemical features of NE in the VTA and DA in the NAc shell evoked by electrical stimulation of noradrenergic neurons in the LC are characterized using in vivo dual-microelectrode fast-scan cyclic voltammetry (FSCV). DA and NE release evoked by the LC stimulation was measured simultaneously in the target brain regions with two separate carbon-fiber microelectrodes, which allows direct comparison of their dynamic process and drug effects on them in same preparation. Here, we present that FSCV enables monitoring fast events of NE in the VTA and demonstrate that DA in the NAc shell evoked by electrical stimulation of the LC is regulated by alpha 1- and alpha 2-adrenergic receptors. This study will potentially help future investigations on the role of LC-NE system in modulating the dopaminergic neurons and its related behaviors.

**Keywords:** Bioanalytical, Electrochemistry, Neurochemistry

**Application Code:** Neurochemistry

**Methodology Code:** Electrochemistry
The quantitative determination of glucose, which is an important metabolite for living organisms, is based on the major investigations in the point of clinical diagnostics of diabetes since it is a world-wide public health problem and one of the leading causes of death and disability in the world [1]. Enzyme-modified electrodes have been mostly applied for this purpose by the scientific researchers [2]. However they have some disadvantages, such as a lack of stability, the high cost of enzymes, the need of complicated immobilization procedures, and critical operating conditions [3]. Therefore, for several years, in order to overcome these problems considerable attention has been paid and there is an ever-growing demand to create electrochemical glucose sensors with high sensitivity, high reliability, short response times, good reproducibility and repeatability, and low cost, especially non-enzymatic amperometric biosensors, which are currently the most popular.

In this study, 1,10-phenanthroline-5,6-dione-modified glassy carbon (PD/GC) electrode surfaces were prepared via electrochemical oxidation of 1,10-phenanthroline-5,6-dione (PD) at the glassy carbon (GC) electrode by cyclic voltammetry technique. Electrochemical studies were performed using three-electrode cell system. For this aim, the carbon electrode was used as a working electrode, platinum wire was used as a counter electrode, Ag/Ag+ was used as a reference electrode. The modified electrode surface prepared in this way was characterized by cyclic voltammetry and electrochemical impedance spectroscopy techniques in the presence of redox probes and the obtained results were compared with the results of bare GC surface as described in our previous studies [4,5]. The PD/GC electrode surface allows highly selective and sensitive, stable and fast potentiometric sensing for glucose, which is promising for the development of non-enzymatic glucose sensor.

Keywords: Electrochemistry, Electrode Surfaces, Electrodes
Application Code: Bioanalytical
Methodology Code: Electrochemistry
In our previous research works we have proposed enzymatic synthesis of conducting polymer layers [1] and conducting polymer based nanoparticles [1-6], [7-8]. Polypyrrole [1-5,7,8] and polyaniline [6] based nanoparticles were synthesized by this method up to this moment. Good biocompatibility of conducting polymer polypyrrole nanoparticles [7,8], encourages us for some biomedical applications of these nanoparticles. Therefore some advanced studies of these nanoparticles were performed.

Dynamic light scattering was applied in order to investigate changes of diameter of synthesized polypyrrole and polyaniline nanoparticles during polymerisation course. It was determined that during the polymerisation course the size of polymeric nanoparticles increases gradually. Evaluation of enzymatic activity of entrapped enzyme glucose oxidase (GOx) E.C. 1.1.3.4. from Penicillium vitale and from Aspergillus Niger was evaluated electrochemically and spectrophotometrically. It was demonstrated that enzymatic activity is reversibly dependent on duration of polymerisation reaction. Biocompatibility tests in order to determine biocompatibility of polypyrrole nanoparticles with steam cells and mice were performed.

Acknowledgement
This project was financially supported by Lithuanian Scientific Council.

Keywords: Electrochemistry, Spectroelectrochemistry, Spectrophotometry
Application Code: Nanotechnology
Methodology Code: Electrochemistry
Cloud point extraction is a well-established technique for the preconcentration 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, little is known about the suitability of cloud point extraction for electroanalytical methods, such as stripping voltammetry. We have developed a cloud point extraction procedure for the determination of cadmium (Cd2+) by anodic stripping voltammetry (ASV) with a glassy carbon (GC) working electrode. This extraction method was performed without the use of added chelating agents, but using iodide to neutralize the charge on Cd2+ by forming CdI2. This novel combination achieved a detection limit of 25 ppb. Applicability of the procedure to ground 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, Metals, Voltammetry

Application Code: Environmental

Methodology Code: Electrochemistry
This work describes the development, results, and uncertainty of assay procedures used in assigning the reductometric assay for sodium oxalate, a standard for oxidation-reduction reactions certified by the National Institute of Standards and Technology (NIST).

Sodium oxalate is commonly used to standardize potassium permanganate. Although the titration of sodium oxalate with potassium permanganate can provide a very precise result, it is subject to bias depending on factors such as the temperature, acid strength, mixing, rate of titrant addition, and the endpoint detection method.

Sodium oxalate can be assayed precisely by titration against ceric ammonium nitrate. Ceric ammonium nitrate is commercially available as a primary standard; however, that does not provide traceability to the SI. Assay procedures for ceric ammonium nitrate based on coulometry and by titrimetry against arsenic trioxide are described.

The advantages and disadvantages of candidate titrimetric assays will be discussed. Titration of sodium oxalate against ceric ammonium nitrate can be performed in a solution of nitric or perchloric acid. The endpoint can be detected visually using an indicator (ferroin) or potentiometrically (platinum electrode) and as a direct titration or a back titration (with ferrous ammonium sulfate).

Keywords: Chemical, Quantitative, Titration, Wet Chemical Methods
Application Code: Environmental
Methodology Code: Electrochemistry
The recently developed, fully reversible polymeric membrane based sensor for polyion determination, where polyion flux is controlled instrumentally over a 3 step protocol, was used as a detector in flow-injection systems for monitoring enzymatic reactions. Coupling of flow-injection analysis (FIA), the most widely used method for achieving high-throughput analysis of target analytes, with polyion sensitive pulstrodes can result in a powerful analytical tool.

In our work we have focused on monitoring enzymatic degradation of polyions in flow injection mode. Protamine and serine protease trypsin were used as a model system. A polymeric membrane containing a lipophilic inert salt of the form R+R- (where R+ and R- are tridodecylmethylammonium (TDMA+) and dinonylnaphthalene sulfonate (DNNS-), respectively) with no intrinsic ion-exchange properties was used to detect polyion species. A galvanostatic pulse was applied for 1 s with fixed magnitude, to polarize the membrane and induce a polyion flux. The membrane was then subjected to an open-circuit potential for 0.5 s, and finally a 0 V vs. reference electrode potentiostatic pulse (> 8 s) was applied to return the sensor to its initial state. Fully reversible and reproducible electromotive force responses in the flow system were achieved. Enzyme activity was determined in two flow arrangements; (1) by incubation of enzyme with substrate and then subsequent injection into flow and (2) by injection of enzyme solution into the stream containing substrate where incubation time was controlled by tubing length and flow rate.

This work has been supported by the European Union in the framework of Regional Development Fund through the Joint UW and WUT International PhD Program of Foundation for Polish Science – “Towards Advanced Functional Materials and Novel Devices” (MPD/2010/4).

**Keywords:** Enzyme Assays, Flow Injection Analysis, Sensors

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
The ability to make rapid electrochemical measurements of neurotransmission from living cells is desirable for the monitoring the changes in concentrations of neurotransmitters in real time. In this work, Chemical Vapor Deposition (a rapid approach to producing high purity semiconductors) is used to synthesize thin film polymer electrodes composed of poly(3,4-ethylenedioxythiophene):tosylate (PEDOT:tosylate). This reaction proceeds via the acid-catalyzed radical polymerization of 3,4-ethylenedioxythiophene using ironIII tosylate as the radical initiator and pyridine as a weak base to control the reaction rate. Similar films have been synthesized previously but high capacitances have results in large time constants which limits rapid electrochemical measurements. By varying the ratios of these precursors as well as the reaction temperature it is possible to control the thickness of the deposited polymer film. Films can be synthesized with thicknesses varying from 40-500 nm as measured by atomic force microscopy and optical transmittance. A low-pressure microwave-generated plasma was used to dry etch the films forming patterned electrodes with micrometer scale features. The resulting PEDOT:tosylate electrodes were electrochemically characterized using steady-state cyclic voltammetry and were found to have capacitances lower than 400 µF/cm$^2$. The utility of conductive polymer electrodes with low capacitance is demonstrated by performing fast-scan cyclic voltammetry of redox active neurotransmitters.
Surface plasmon enhanced light absorption and local field intensity can benefit the light photocatalytic system by improving its light absorption efficiency while maintaining a short carrier transport distance. In this presentation, experimental evidences of such enhancement process will be discussed for \( \text{Fe}_2\text{O}_3 \) thin film electrode modified with Au nanorods (NRs) in a top configuration. Au NRs are self-assembled onto a hematite thin film electrode using electrostatic force between their positively charged cetyltrimethylammonium bromide (CTAB) layer and negatively charged hematite surface functionalized with poly (4-styrenesulfonate). The photoelectrochemical reaction of the plasmon active substrate for water oxidation reaction is performed and compared at various hematite layer thicknesses. The significant increase in photocurrent in the region of surface plasmon absorption is attributed to the enhanced visible light absorption of \( \text{Fe}_2\text{O}_3 \) in the presence of the plasmon active Au NRs. On-going research with standing Ag nanowire arrays prepared on transparent substrate will be described for potential application in such surface enhanced photocatalytic reaction.

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

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

**Methodology Code:** Electrochemistry
Abstract Text
The fabrication of effective surface-enhanced Raman scattering (SERS) substrates has been the subject of intensive research because of their useful applications. Dendritic gold (Au) rod (DAR) structures prepared by simple one-step electrodeposition in a short time were examined as an effective SERS-active substrate. The SERS activity of the DAR surfaces was compared to that of other nanostructured Au surfaces with different morphologies, which revealed that highly faceted sharp edge sites present on the DAR surfaces play a critical role in inducing a high SERS activity. The DAR surfaces exhibit excellent SERS enhancement reproducibility and can be effectively utilized in electrochemical SERS systems. The DAR surfaces were further modified with ultra-thin layers of Pd and Pt, which also exhibited high SERS activity. The Pd or Pt layers on DAR surfaces showed a reversible SERS behavior for pyridine adsorbed on these surfaces during the cycling to cathodic potential regions. We examined the electrochemical oxidation of formic acid on Pt/Pd layers on DAR by in-situ SERS measurements, from which different mechanistic features between Pt and Pd surfaces during the electrooxidation of formic acid were revealed. The SERS-active metal surfaces presented in the present study can be used for in-situ examination of other important electrochemical reactions.

Keywords: Electrochemistry, Electrode Surfaces, Spectroelectrochemistry, Surface Enhanced Raman
Application Code: Materials Science
Methodology Code: Electrochemistry
Biomorphic calcium phosphate (CaP) microspheres with hierarchical porous structure were successfully synthesized using natural cole pollen grains as templates and were further employed for horseradish peroxidase (HRP) immobilization. Scanning electron microscopy and Fourier transformed infrared spectroscopy analysis revealed the porous structure of the obtained CaP microspheres and effective immobilization and conformation retention of HRP on CaP. The immobilized HRP displayed direct, reversible, and surface-controlled redox reaction with electron transfer rate constant of $1.96 \text{ s}^{-1}$. Furthermore, the immobilized HRP exhibited high sensitivity to the reduction of $\text{H}_2\text{O}_2$ with a linear range from $5.00 \times 10^{-9}$ to $1.27 \times 10^{-6}$ M with the sensitivity of $30357 \text{ u A mM}^{-1}\text{ cm}^{-2}$. The detection limit, based on a signal-to-noise ratio of 3, was down to $1.30 \times 10^{-9}$ M. The apparent Michaelis–Menten constant ($K_{\text{M}}^{\text{app}}$) of the immobilized HRP was $0.92 \text{ u M}$. Thus CaP with hierarchical porous structure has significantly promoted the direct electron transfer between HRP and electrode and improved the affinity between HRP and $\text{H}_2\text{O}_2$. It is an attractive material for application in the immobilization of proteins and construction biosensors.

We greatly appreciate the support of NSFC (21075107, 21275124, and 21275125), Foundation of Jiangsu Educational Committee (12KJB150022), and the project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions and the Qinglan Project of JiangSu Province.

Keywords: Analysis, Biosensors, Electrodes, Materials Science
Application Code: Materials Science
Methodology Code: Electrochemistry
Alkaline metal ion (secondary) batteries store energy reversibly by shuttling ions between anode and cathode materials capable of electrodepositing them or providing a host for their intercalation/deintercalation. While the performance characterization of these electrodes at the macroscale is common, very little is known about the rates and impact of structural and reactive heterogeneity at the micro- and nano-scale. For instance, extensive discharging and repeated cycling results in increased overpotentials and decreased total charge capacity of anode electrodes. The spatial distribution of these losses and how they relate to structural defects with unequal reactivity is unknown. Understanding and visualizing these mechanisms is therefore of paramount importance to battery development.

In this study, an amperometric ion-sensitive platform is introduced. A micropipette probe was brought into close proximity to a carbon substrate onto which controlled defects had been previously generated. The bulk solvent system was modeled after that of extant commercial batteries. By collecting alkaline ions at the probe, deintercalation processes at the carbon substrate were measured quantitatively and with spatial resolution. The current response served as a direct measure of battery cyclability. Furthermore, by coupling the described configuration with a scanning electrochemical microscope (SECM), this study obtained electrochemical maps of ion deintercalation, which can be correlated to defects and structures with dissimilar electron transfer kinetics. This technique provides an unprecedented analysis of anode degradation, accessing details that are often lost in conventional electrochemical methods.

Keywords: Electrochemistry, Electrode Surfaces, Microelectrode, Sensors
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Electrochemistry
Abstract Text

Simple and cost effective analytical techniques capable to determine trace concentration levels of high-molecular weight polycyclic aromatic hydrocarbons (HMW-PAHs) still remains a challenge. The main problem that confronts the chromatographic analysis of HMW-PAHs is the large number of isomers with very similar elution times and mass fragmentation patterns. Research efforts in our group have focused on laser excited time-resolved Shpol'skii spectroscopy (LETRSS). Unambiguous determination of dibenzo[a,l]pyrene, dibenzo[a,e]pyrene, dibenzo[a,i]pyrene, dibenzo[a,h]pyrene and naphtho[2,3-a]pyrene was made possible on the basis of spectral and lifetime information recorded from frozen solutions in n-alkane solvents. The research presented here investigates the effect of the n-alkane solvent on the LETRSS analytical figures of merit of HMW-PAHs. Depending on the PAH, the narrowest full-width half maximum and the highest fluorescence intensities were obtained either with n-octane, n-nonane or n-decane. The improvement of limits of detection provided trace determination at the parts-per-trillion concentration level with micro-liters of organic solvent.

Keywords: Environmental, Fluorescence, Laser, PAH
Application Code: Environmental
Methodology Code: Fluorescence/Luminescence
**Session Title**: Fluorescence/Luminescence/UV-VIS Bio and Nano

**Abstract Title**: Low-Temperature Synchronous Fluorescence Spectroscopy with Fiber Optic Probes for the Analysis of High Molecular Weight Polycyclic Aromatic Hydrocarbons

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**Abstract Text**

We present the first report on the analytical potential of 4.2 K synchronous fluorescence Shpol’skii spectroscopy for the analysis of high-molecular weight-polycyclic aromatic hydrocarbons (HMW-PAHs). An instrumental set-up for collecting low-temperature fluorescence data was developed in our lab, which couples a homemade bifurcated fiber optic bundle to a commercial spectrofluorometer. The new set-up facilitates the collection of synchronous fluorescence data at liquid cryogen temperatures (77 K and 4.2 K). The optimization of wavelength offsets permits the successful determination of dibenzo[a,l]pyrene, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, and naphtho[2,3-a]pyrene without previous chromatographic separation. The feasibility of this approach is demonstrated with the analysis of a soil extract with a complex matrix composition. Spectral characteristics and 4.2 K analytical figures of merit are presented for the above mentioned HMW-PAHs.

**Keywords**: Environmental/Soils, Fiber Optics, Fluorescence, PAH

**Application Code**: Environmental

**Methodology Code**: Fluorescence/Luminescence
Bacterial endotoxin (BE) is one of the bacterial toxins from the outer membrane of gram-negative bacteria. BE causes fever or shock when it enters the human blood. BE concentration in water for pharmaceutical and dialysis purposes is strictly controlled. BE test is used to quantify endotoxins using limulus amebocyte lysate. Three existing techniques for quantifying endotoxins include the gel-clot technique, based on gel formation; the turbidimetric technique, based on the development of turbidity after cleavage of an endogenous substrate; and the chromogenic technique, based on the development of color after cleavage of a synthetic peptide-chromogen complex. These techniques are agreed upon by Pharmacopoeia of the United States of America, European Pharmacopoeia, and Japan Pharmacopoeia. However, the abovementioned techniques have some limitations such as prolonged measurement time. These photometric quantitative techniques, turbidimetric and chromogenic techniques, take 50–90 min in order to measure 0.001 EU/mL. Hence, a rapid and high sensitivity measurement technique is desired. The bioluminescence technique allows rapid measurement (less than 25 min) at 0.0005–0.01 EU/mL and high sensitivity (detection limit 0.0003 EU/mL). A genetically modified luciferase generates luminescence intensity at least 10-fold higher than that generated by wild-type luciferase. This new technique also meets the Pharmacopoeias’ criteria. The correlation coefficient of the standard curve at 0.0005–0.01 EU/mL is 0.99 and the endotoxin recovery test for interfering factors at middle concentration of the standard curve is within 100–150%.
Molecular imprinting is a simple method to prepare artificial receptors with high affinity and selectivity for template molecules. Molecularly imprinted polymers (MIPs) have been prepared by copolymerization of a functional monomer(s) and a cross linker(s) in the presence of a template molecule, followed by removal of the template to create binding cavities. In recent years, protein imprinting has been studied as a strategy to produce artificial protein receptors. However, since most of MIPs for proteins were prepared in solution, thermal motion of proteins would be obstacle for construction of the highly ordered binding cavities bearing high selectivity. Moreover, after the removal process, unremovable template molecules may remain inside polymer, resulting in the decrease of binding cavities.

In this study, transcription-type protein imprinting was performed by using protein-immobilized glass substrate, where biotinylated template protein cytchrome c (Cyt) was attached to the avinylated nanoparticles immobilized on the glass substrate. Radical polymerization was performed in the space between the protein immobilized glass substrate and vinyl group modified glass substrate in the presence of a crosslinker and a functional monomer. This transcription-type imprinting method creates MIPs having binding cavities only on the surface after the removal of the protein-immobilized glass substrate. Affinity of MIPs to Cyt and reference proteins were analyzed in a competitive manner with FITC-labeling Cyt using a fluorescent microscope. The fluorescence intensity decreased with increasing concentration of free Cyt. For reference proteins, less fluorescence change was observed, indicating that MIPs having selective binding property for Cyt can be prepared by the proposed method.
For contemporary analytical chemistry micro- and nanospheres are interesting materials to explore in search for sensors of improved proprieties. 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 polymer, e.g. Poly(maleic anhydride-alt-1-octadecene), as starting material. Depending on synthesis procedure, the size of nanospheres obtained is ranging from about 120 nm to 250 nm. The spheres synthesized were used as fluorimetric ions-nanosensors using an optrode approach. Thus obtained pH fluorimetric sensors were characterized by fast responses over broad pH range. The potassium ion sensors prepared (as receptor valinomycyn was used and chromoionophore as optical transducer) where showing reproducible linear dependence of fluorimetric signal on change of logarithm of potassium ions concentration in solution within the range from 10^-5 M to 10^-1 M. The unique linear dependence obtained was attributed to participation of negatively charged surface groups of nanosphere in ion-exchange. Additional advantage arises from possibility of modification of spheres surface due to covalent attachment of a reference dye.

Keywords: Fluorescence, Nanotechnology, Sensors
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Cyclophanes have a potential to incorporate organic molecules by their cavities and function as molecular recognition elements. Previously, we reported anthracenophanes composed of two L-lysine methyl ester as the linkers, which were prepared via self assembling-based thermodynamically controlled intermediate.[1] In this study, the lysine methyl ester-linked anthracenophane was hydrolyzed to obtain a water-soluble anthracenophanes bearing free carboxylic acids, and examined pH-responsive optical properties of the anthracenophane.

A change in the fluorescence properties and the conformation of the anthracenophane by pH change was evaluated by 2D-NMR and fluorescence spectrophotometer. It was found that the anthracenophane has three different conformations depending upon their charge balance, derived from the charge of the functional groups on the linker moieties, and fluorescence of anthracenophane was quenched in basic conditions. A quenching mechanism was investigated by methylation of -amino groups on the L-lysine linker, suggesting that the phenomenon may be caused by photo-induced electron transfer of the lone pair of electrons on the amino groups of the water-soluble anthracenophane. Molecular recognition and signal transduction abilities as a molecular sensor was also investigated.


Keywords: Bioanalytical, Fluorescence
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Metalloporphyrins capable of the coordination ability can act as fluorescence sensors, in which the binding events can be transduced into spectral change. Polymerizable porphyrins were also reported and by using them optical sensor materials were prepared. Since porphyrins possess low hydrophilicity, there is a limitation for the use in aqueous solution. Polyethylene glycol(PEG)-linked metalloporphyrins have been studied and confirmed that short-chain PEGs couldn’t give enough hydrophilicity and long-chain PEGs disturbed interaction with porphyrins and target molecules. Therefore, in this study, we designed and synthesized a novel polymerizable amphiphilic metalloporphyrin linked with glycerol dendron G3 (GD-G3) bearing polyether structure. We examined the hydrophilicity, binding property, amphiphilicity, and surface-active ability of the porphyrin monomer, and then prepared the nanoparticles by co-polymerizing the porphyrin monomer with styrene in aqueous media. Because of the amphiphilic property, the orientation of porphyrin moiety may be ordered around surface of nanoparticles in aqueous solution. In this presentation, we discuss the characteristics of the amphiphilic porphyrin-based functional nanoparticles in detail.

**Keywords:** Bioanalytical, Fluorescence, Protein, Sensors  
**Application Code:** Bioanalytical  
**Methodology Code:** Fluorescence/Luminescence
Fluorescence/Luminescence/UV-VIS Bio and Nano

Novel Coelenterazine Derivatives for Bioluminescence Applications

Bioluminescence (BL)-based assays such as gene reporters in cell cultures and \(\textit{in vivo}\) imaging have the lowest background and high sensitivity, because in contrast to fluorescence, BL does not require an excitation light source. Most of the investigated BL systems require a BL substrate (luciferin) and an enzyme (luciferase) to emit light.

The most useful type of luciferin for imaging is the firefly luciferin, because it emits at relatively long-wavelength with the brightest intensity among all BL substrates. But for light emission, firefly luciferin requires the presence of Mg\(^{2+}\) and ATP as co-factors, which may lead to complex protocols when applied to bioanalysis.

Recently, \(\textit{Renilla}\) luciferase (Rluc) with coelenterazine (CTZ) as the substrate is used as versatile reporter protein, because of its independence of the presence of Mg\(^{2+}\) and ATP. So far, a large number of CTZ derivatives have been reported to extend its application. For example, one of the CTZ derivatives, the blue-shifted DeepBlue\(^{\text{TM}}\) is used as BL resonance energy transfer (BRET) donor for the green fluorescent protein (GFP), because it emits around 400 nm with minimal interference with the emission of the GFP acceptor. Therefore, DeepBlue\(^{\text{TM}}\) is applied for BRET studies, such as protein-protein interaction imaging.

In this research, novel CTZ derivatives (6-pi-X-CTZ, X = H, OCH\(\textsubscript{3}\), OH, Phenyl and CF\(\textsubscript{3}\)) have been developed (Fig. 1). These CTZ derivatives showed BL with the known Rluc variant, Rluc8.6. As a result, all 6-pi-X-CTZ (except for 6-pi-OH-CTZ) showed an approximately 40 nm blue shifted emission compared to CTZ. On the other hand, 6-pi-OH-CTZ showed an approximately 50 nm red shifted peak. The BL intensities of 6-pi-H-CTZ, 6-pi-OCH\(\textsubscript{3}\)-CTZ, 6-pi-Phenyl-CTZ and 6-pi-CF\(\textsubscript{3}\)-CTZ are 14%, 81%, 44%, 94% and 1.4% of that of CTZ with Rluc, respectively. Furthermore, 6-pi-H-CTZ showed 22.9-fold stronger BL intensity than that of DeepBlue\(^{\text{TM}}\). Thus 6-pi-H-CTZ is useful as a bright blue-shifted CTZ derivative, which can be used as an alternative to DeepBlue\(^{\text{TM}}\).

Keywords: Bioanalytical, Chemiluminescence, Luminescence
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Despite recent advances in treatment for breast cancer, earlier detection of disease in a more sensitive and less invasive manner could improve patient care and outcome. Mammography is a powerful imaging technique, but it is associated with significant numbers of both false negatives and false positives, which can result in disease progression or overtreatment, respectively. Current serum based biomarkers used for disease monitoring in breast cancer, such as cancer antigen 15-3 and carcinoembryonic antigen, lack both the sensitivity and specificity required for early detection. Protein biomarkers that have the potential to indicate earlier diseased states likely exist in serum at concentrations below the detectable limits of standard methods, such as ELISA. Our main goal is to use the ultrasensitive single molecule arrays (SiMoA) technique developed in our laboratory to detect a fingerprint of biomarkers associated with breast cancer within patient serum. The on-bead approach of SiMoA decreases the LOD of traditional ELISA by 100-1000 fold by employing a digital counting methodology. Our method potentially offers a much less invasive option to mammography and may enable both a more sensitive and less invasive technique for earlier detection of breast cancer. This work describes our current progress toward developing SiMoA assays for several biomarkers of interest as well as preliminary results from mouse models. Using mouse xenographs and human in mouse models we can correlate tumor size with protein concentrations within the serum. This work was funded by DOD BC100510 (W81XWH-11-1-0814).
Polymeric microsphere-based ion optodes were fabricated via a solvent displacement process on the basis of bulk optode theory and inner filter effect. Plasticized poly(vinyl chloride) along with the optode components and upconverting nanorods was dissolved in a solvent miscible with water. The polymer solution was rapidly injected into a stirred aqueous phase containing a surfactant (e.g. an amphiphilic polymer) that caused spontaneous emulsification. The resulting microspheres were doped with upconverting nanorods.

The microsphere-based sensor exploits the phenomenon of upconversion luminescence of hexagonal-phase NaYF4: Yb, Er nanocrystals that can be excited with 980-nm laser light to give a green and red emission, together with absorbing pH-sensitive dye that causes an inner filter effect. The sensor itself is pH-dependent, and can also respond to different ions when both ion-exchanger and ionophore are added. It is promising for performing measurements in plasma or even strongly coloured sample.

Keywords: Analysis, Fluorescence, Ion Exchange, Particle Beam
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Prostate cancer is the second leading cause of death occurred in men, which is likely due to the difficulty in perceiving the early signs and symptoms. While prostate-specific antigen (PSA) test is widely used to screen men for prostate cancer, PSA level in blood may rise with age and a number of benign conditions. We herein designed and developed a genosensor based on duplex-specific nuclease (DSN)-assisted amplification for the detection of prostate cancer-specific microRNA (PCS-miR) to achieve better sensitivity and specificity. CdSe/ZnS core/shell quantum dots (QDs) were firstly modified with a DNA probe comprising a recognition region for PCS-miR along with a quencher molecule tagged on the end. In the presence of PCS-miR, the hybridization between the probe and PCS-miR could be recognized by DSN, followed by the digestion of DNA in the DNA:RNA duplex. This led to the release of PCS-miR and recovery of fluorescence signal from QDs, resulting in a turn-on fluorescent genosensor. Our results confirmed that this sensing platform yielded picomolar detection limit along with high specificity. Preliminary results also show that this genosensor sustains its performance in analyzing serum samples and holds great promise to be applied in clinical diagnostics.
Sodium dodecyl sulfate (SDS) is an anionic detergent, commonly used in molecular biology and protein chemistry for its denaturing and solubilizing characteristics. It is especially useful for dissolving membrane proteins, recombinant proteins and other proteins that have a large degree of hydrophobic characteristics. One quantitation method currently in use is based on the use of a dye, Stains-all, the color of which changes from intense fuchsia to yellow upon addition of and binding to SDS. The robust and reproducible nature of this test method led us to assess whether other detergents could be similarly quantified.

Sodium laureth sulfate and N-lauroylsarcosine are anionic detergents, which are commonly used as low concentration surfactants in in-vitro diagnostics. Differences in percent concentration may affect reagent stability, immunoreactivity and background signals. These detergents are often supplied as solutions, therefore, quantitation of these detergents may prove useful at purchase or in final use formulation.

Lauroyl-l-glutamate is an amino-acid based detergent, n-dodecylphosphocholine (Fos-choline-12) is an ionic phospholipid, both of which are used as solubilizing agents for refolding of insoluble proteins and antibody fragments. A targeted percentage is used during solubilization, and precise concentration may be critical to the success of the process.

We adapted the SDS quantitative method to quantitate these detergents, by examining parameters such as wavelength, diluent buffering agent, pH, and standard curve concentrations.

Keywords: UV-VIS Absorbance/Luminescence
Application Code: Bioanalytical
Methodology Code: UV/VIS
C-reactive protein (CRP) is one of biomarkers to indicate inflammation, as CRP increases approximately 1,000 times when inflammation happened in a human body. More recently, CRP is considered as a biomarker for arterial sclerosis at low concentration range. Until now, CRP sensing was carried out by immunonephelometry or enzyme-linked immunosorbent assay (ELISA). These methods are required to use biological antibodies and enzymes, however, such molecules were expensive and chemically unstable. Therefore, sensing systems using artificial molecular recognition materials are attracted attentions. Au nanoparticles are useful materials for sensitive sensing systems because visible light was extincted at the surface of Au nanoparticles due to the localized surface plasmon resonance (LSPR) property and the wavelength was changed sensitively when the permittivity around the Au nanoparticles was changed. In this study, in order to develop sensitive sensing systems for CRP based on the LSPR property of Au nanoparticles, we synthesized poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC)-grafted Au nanoparticles by surface-initiated controlled/living radical polymerization in dispersed systems. PMPC is well-known for modifying the surface for resistance of adsorption of most proteins, however, phosphorylcholine is also known as a selective ligand for CRP. The CRP sensing can be carried out only using the UV-vis spectroscopy and the extinction wavelength change due to the CRP adsorption on the PMPC layer was measured.

**Keywords:** Bioanalytical, Nanotechnology, Protein, UV-VIS Absorbance/Luminescence

**Application Code:** Bioanalytical

**Methodology Code:** UV/VIS
Fluorescence/Luminescence/UV-VIS Bio and Nano

Legionella Pneumophila Detection by rRNA

The current procedure used to detect and identify the Legionella species takes a lot of time and requires several steps; therefore, new methods for the detection of Legionella are of great interest. A modern detection target is rRNA, which is more numerous than DNA, and is only present in living cells. A new sandwich hybridization test is performed on a microtiter plate and takes less than 2.5 hours. Cell count quantification is possible with this kit using photometric methods. Compared to PCR, this system does not count dead cells, is much easier to use, is less expensive, and is not affected by the sample matrix. The rapidity, sensitivity, reliability, robustness, adaptability to sample matrix, and time savings meet today’s analytical microbiology demands.

Specificity is achieved by targeting conserved or unique rRNA sequences. A biotin-labeled capture probe is used to immobilize the target sequence on a solid support plate (streptavidin-coated microtiter plate). A digoxigenin labeled detection probe provides an enzyme linked optical signal read-out. Detection results from application of anti-DIG-horseradish peroxidase Fab fragments. The bound complex is visualized by horseradish peroxidase substrate TMB. Photometric data are measured at 450 nm and compared with standard solutions.

Keywords: HPLC, HPLC Columns, SPME, Titration
Application Code: Environmental
Methodology Code: Other (Specify)
Sensitive molecular target analysis is pivotal in forensic field and disease theranostics. Signal amplification is key to sensitive molecular analysis, especially for the detection of trace crime-related targets or early disease diagnosis. Enzyme-free and isothermal signal amplification is appealing for the development of low-cost onsite molecular analysis and point-of-care diagnosis. Here we report enzyme-free, isothermal and sensitive detection of molecular targets based on target-induced assembly of DNA circuits via Catalyzed Hairpin Assembly (CHA), a catalytic reaction with signal-amplification capability. Specifically, by rational design, we cut a target-specific aptamer into two parts, termed as split aptamers. A catalytic DNA strand (C) that can initiate CHA was tethered with one of the split aptamers and originally locked by a third DNA strand (L). The presence of target induced the re-integration of split aptamers, replacing L, and subsequently activating C to initiate CHA. C was recycled to continuously catalyze CHA, resulting in signal amplification and dramatic enhancement of fluorescence signal intensity. Cocaine was used to demonstrate this principle, and the low-cost yet efficient and sensitive cocaine detection is extremely significant in many forensic or biomedical scenarios. Our results showed that this sensor can detect cocaine with high sensitivity, with the limit of detection of 1 nM. To expend the feasibility of this sensor for practical applications, we are working on cocaine detection in biological fluids, such as blood, saliva, and urine. The inherent modularity of the DNA circuit allowed us to readily adapt our sensor to detect many other molecular and cellular analytes, and this system can also be applied to many other analytical formats (e.g., colorimetric and electrochemical signaling). Overall, this demonstrates an enzyme-free and isothermal approach to sensitive target analysis.

Keywords: Biosensors, Detection, Fluorescence, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Since the discovery of its antiproliferative abilities, cisplatin has been a staple in chemotherapies. Its ability to induce apoptosis by forming adducts with DNA has made it a choice of treatment for 50–70% of all patients with cancer. Current dosing methods utilize either a single dose across patient classes or body surface area dosing to avoid the associated neuro- and nephrotoxicity, with the optimal concentration in sera being between 0.1 and 10 [micro]M. However, these initial doses may be ineffective and must often be increased over a period of several weeks to months. This process poses severe harm to patients with the most aggressive cancers or those with compounding factors. This, in turn, necessitates a rapid method for detecting cisplatin in sera on site so that clinicians may better adjust the dosage of chemotherapy within hours. We have developed a fluorescence-based method for detecting free cisplatin in sera using a platinum-catalyzed Tsuji-Trost reaction. After protein precipitation, human serum was spiked with cisplatin to create a standard curve. The standard curve showed a linear relationship between concentration of cisplatin and change in fluorescence intensity. A linear regression then allowed for the determination of cisplatin concentrations in unknown samples. With this method, we have achieved a limit of detection of 68 nM without the chromatographic techniques that other methods require. Using a double-blind study, we have also determined the concentration of cisplatin in unknown samples within 30% of the true value.

Abstract Text

Since the discovery of its antiproliferative abilities, cisplatin has been a staple in chemotherapies. Its ability to induce apoptosis by forming adducts with DNA has made it a choice of treatment for 50–70% of all patients with cancer. Current dosing methods utilize either a single dose across patient classes or body surface area dosing to avoid the associated neuro- and nephrotoxicity, with the optimal concentration in sera being between 0.1 and 10 [micro]M. However, these initial doses may be ineffective and must often be increased over a period of several weeks to months. This process poses severe harm to patients with the most aggressive cancers or those with compounding factors. This, in turn, necessitates a rapid method for detecting cisplatin in sera on site so that clinicians may better adjust the dosage of chemotherapy within hours. We have developed a fluorescence-based method for detecting free cisplatin in sera using a platinum-catalyzed Tsuji-Trost reaction. After protein precipitation, human serum was spiked with cisplatin to create a standard curve. The standard curve showed a linear relationship between concentration of cisplatin and change in fluorescence intensity. A linear regression then allowed for the determination of cisplatin concentrations in unknown samples. With this method, we have achieved a limit of detection of 68 nM without the chromatographic techniques that other methods require. Using a double-blind study, we have also determined the concentration of cisplatin in unknown samples within 30% of the true value.

Keywords: Biological Samples, Drugs, Fluorescence, Trace Analysis
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
The development of chemometric methods has substantially improved the quantitative usefulness of the fluorescence excitation-emission matrix (EEM) in the analysis of dissolved organic matter (DOM). Due to the fact that the fluorescence of DOM can be quenched when it is bound to certain metal ions, a study was conducted to explore the application of regional integration analysis (RIA) to fluorescence quenching behavior. Another widely used method, parallel factor analysis (PARAFAC), was compared with RIA to validate the applicability of RIA. Three fulvic acids including soil fulvic acid (SFA), Oyster river fulvic acid (ORFA) and Suwannee river fulvic acid (SRFA) were used in this study. The EEM spectra obtained were divided into five regions according to fluorophores and two distinct peaks were observed in humic acid-like and fulvic acid-like regions. Fluorescence information for these two regions was calculated by the RIA method. Stability constants and other parameters were obtained for uranyl ion (UO$_2^{2+}$) using the Ryan-Weber fluorescence quenching model. Results indicated that binding ability ($K$) follows the order SFA > ORFA > SRFA while binding site concentration ($C_L$) followed the reverse order SRFA > ORFA > SFA. Comparison of RIA and PARAFAC data treatment methods showed good agreement (less than 4 % difference in log $K$ values). Our results demonstrated the applicability of the RIA method for fluorescence quenching studies as well as its great potential in quantitative fluorescence analysis, whose application can be extended to monitoring natural fresh waters and other complicated fluorescent substances.
A Preliminary Investigation of the Effects of Metal Ions on the Fluorescence of Known Iron (II) Chelators: Analytical Utility for Determination of Iron

In this paper, the effect of binding of iron(II) and other selected ions on the fluorescent properties of known Fe(II) chelating agents, such as 1,10-phenanthroline, 3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine disodium salt (Ferrozine), and 3-(2-pyridyl)-5,6-difurysulfonic acid-1,2,4-triazine disodium salt (Ferene S), will be investigated and presented. The paper will focus mainly on results obtained for iron. The aforementioned Fe(II) chelators, and their many analogues, are well known for their high selectivity for Fe(II) and formation of high-molar-absorptivity Fe(II) chelates for the sensitive analytical determination of iron by visible spectrophotometry. These molecules are also highly conjugated and possess aromatic rings, thus presenting the possibility of innate fluorescence, which may lead to the use of these chelators for fluorometric determination of iron and potential enhancement of sensitivity for iron versus visible spectrophotometry. The goal of this research is to explore the possibility of fluorescence by these ligands for Fe(II), and from there, study the relationship between fluorescent signal and Fe(II) concentration and then determine the analytical utility of this relationship via calibration curves and analysis of selected samples, such as abandoned mine drainage and other environmentally relevant samples, for iron.

Sample preparation techniques, calibration data and results, and the determination of iron in selected samples by fluorometry and visible spectrophotometry (for comparison), will be presented and discussed, as will future directions for this research.

Keywords: Calibration, Fluorescence, Metals, Spectrophotometry
Application Code: Environmental
Methodology Code: Fluorescence/Luminescence
Infection of human papillomavirus (HPV) has been the leading cause of cervical cancer world-wide, and therefore screening receptor and exploring the detailed mechanism to infect the host cell became essentially important. It was reported that the major capsid protein (L1) mediated primary attachment of viral particles to cells and the extracellular matrix; while the minor capsid protein (L2) was essential for infection with multiple roles. In addition, the basic-sequence-rich peptides at C-terminus of the L1 protein and the N- and C-termini of the L2 protein of HPV have been proved to efficiently bind to the possible receptors on the cell surface, and therefore will be used as model segments for the purpose.

Polyoxometalates (POMs) are an intriguing and distinctive class of inorganic metal-oxygen clusters. Among them, the europium-containing POMs show strong and sensitive luminescence, which makes them attractive as novel biological probes. The strong interaction of it and the basic-sequence-rich peptides of HPV capsid protein induced great enhancement of the POMs luminescence, which was further used to screen the receptors of glycosaminoglycan (GAG) at cell surface. The higher binding affinity of receptor and peptide would rob the peptide from the luminescence pair, and therefore quench the strong luminescence. Therefore, in the present study, we will report the construction of simple and sensitive luminescence pairs based on the basic peptides of HPV capsid proteins and polyoxometalate, and then apply it to the in-vitro receptor screening for virus attachment on cell surface.

References

Key: Bioanalytical, Luminescence, NMR, Peptides
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
The synergistic sensitized spectrofluorimetry for speciation of chromium (Cr(VI)/Cr(III)) in cetyltrimethylammonium bromide (CTAB)/imidazolium bromide tetradecane ionic liquids (ILs) was developed. The analytical procedure was that the fluorescence intensity of salicylfluorone (SAF) could be quenched by Cr(VI) and the fluorescence quenching value \( F = F_{SAF} - F_{Cr(VI)} - SAF \) increased in the medium of CTAB/ILs. The main influence factors on the fluorescence quenching \( F \) were investigated in detail. Under the optimal conditions, the linear range of calibration curve for the determination of Cr(III) was 20.0-240.0 ng/mL and the detection limit was 3.80 ng/mL. The concentration of Cr(III) was calculated by subtracting Cr(VI) from the total chromium which was determined after oxidizing Cr(III) to Cr(VI) by H2O2. The preliminary synergistic sensitized mechanism was discussed with the distribution coefficient and fluorescence quantum yield of SAF. The method has been applied to the speciation analysis of Cr(VI) and Cr(III) in water samples.

**Keywords:** Environmental/Water, Fluorescence, Speciation

**Application Code:** Environmental

**Methodology Code:** Fluorescence/Luminescence
Pipetting of solutions is a common practice in chemical and biological laboratories. Many core types of chemical analyses are based upon analytical techniques involving accurate delivery of liquid components. A common tool developed to deliver these liquid components is the handheld micropipette, as well as automated versions of the same. Handheld and automated pipettors have become commonplace tools, especially used in many biological and pharmaceutical laboratories. While these tools are familiar to many, their performance differences when pipetting different types of solutions are often over-looked and neglected aspects that relate directly to the accuracy and reproducibility of pipetting performance. For example, it is commonly known that water pipettes differently than serum. This presentation will address the difference in performance of a handheld micropipette when dispensing water versus various types of animal and human serum. These differences are quantifiable and can be accounted for through careful experimentation and attention to physical pipetting details. Details on achieving ideal performance when pipetting serum will also be discussed.
Since diode arrays and CCDs became detectors of choice for spectroscopists, common wisdom has been that diode arrays are better for absorbance measurements than CCDs because of the huge number of electrons in each pixel, minimizing the influence of shot noise. Rapidly read CCDs have proven adequate in some laboratory instruments, as one second of averaging CCD readouts at 30 Hz with 16 bit digitization depth gives, approximately, 18 bit dynamic range. In contrast, CMOS webcams and cellcams typically encode their output as JPG files with 8 bit dynamic range. The cameras have from 8 to 12 bit native signal depth. While scientific CMOS promises to compete with CCDs in many applications, the low cost instrument market has, until recently, made do mainly with linear CCD arrays adopted from the flatbed scanner and photocopier markets.

We report how to broaden the use of consumer-grade CMOS cameras by using appropriate grating technology to allow wide dynamic range measurements to be made with low dynamic range detectors. Absorption, reflection, and luminescence measurements over 4 orders of magnitude intensity dynamic range can be obtained from a single exposure with an 8 bit camera. Resolution dynamically trades off with intensity, so that high intensity signals are analyzed with higher wavelength resolution than weak signals. While precision is not as good as is obtainable for, e.g., a diode array or scientific-grade CCD, it is good enough to do useful spectrophotometry and spectrofluorimetry. Key elements are multiple transmission diffraction gratings giving rise to hundreds of spectral orders with differing throughput and dispersion. The operation of such instruments is explained and examples of working curves shown. The inherent ability to self-calibrate in near-real-time reduces the need for dimensional stability, potentially opening new price/performance tradeoffs in instrument design.

Keywords: Multichannel Spectrometry (CCD CID array), Portable Instruments, Spectrometer, UV-VIS Absorbance
Application Code: General Interest
Methodology Code: UV/VIS
It is a big theme in recent times to evaluate the performance of phosphors used in illuminating light sources and display devices with LEDs and/or ELs as they develop and proliferate. In particular, for one of the important evaluation parameters, quantum efficiency, we proposed a measurement method for fluorescent powder samples using an integrating sphere traceable to the light bulb of the spectral irradiance standard supplied by JCSS (Japan Calibration Service System). However, a frequent request these days is to evaluate not only powder samples but also solution samples. So, a measurement system using an integrating hemisphere capable of flexibly handling samples in any forms was constructed. This system comprises a 150 W xenon lamp, a monochromator to the specified excitation wavelength, an integrating hemisphere to measure excitation and fluorescent light at the same time and a spectrometer.

This system also can eliminate “re-excitation” fluorescence to get the actual quantum efficiency values. We consider and report on our discussion of a method to measure phosphor solution samples by utilizing this system and on our verification of the system by evaluating standard phosphor materials.

Keywords: Chemical, Fluorescence, Spectroscopy
Application Code: General Interest
Methodology Code: Fluorescence/Luminescence
Barrier Ionization Discharge detector generates 17.7 eV helium plasma that ionizes almost all compounds except Neon and Helium. Implementation of quartz barrier lowers discharge current, and avoids heating and damaging electrodes. Since BID is a universal detector with sensitivity over 100 times more than TCD, it is an ideal detector for permanent gas and hydrocarbon analysis.

In this work, a 10-port valve is used to backflush water out to vent, followed by a 6-port valve to trap and release permanent gases. Both TOGAS and Natural gas can be analyzed on the same GC platform.

**Keywords:** Fuels\Energy\Petrochemical, Gas Chromatography, GC, GC Detectors

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

**Methodology Code:** Gas Chromatography
Various forms are yttria-stabilized zirconia are used as ionic conductors in solid oxide fuel cells, oxygen sensors, durable coatings, and substrates for high temperature superconducting materials. Highly aluminum doped nanoparticulate Lanthanum Strontium Manganese Oxide (LSAM) synthesized by a solvothermal microwave method is proposed as a novel material that can form a hermetic, high temperature seal with 3% yttria-stabilized zirconia (YTZP) and also act as an electrode. The nature of the nanoparticles of LSAM was investigated by electron microscopy and X-ray diffraction. Electrical conductivity experiments were conducted as a function of temperature and the chemical reactivity between LSAM and YTZP was examined. LSAM was successfully joined to YTZP by a grain boundary sliding deformation process, and LSAM-YTZP joint formation was attributed to plastic deformation at the joint plane. Excellent joint formation was attributed to decreased particle size. LSAM was investigated as a successful ceramic electrode material for use with YTZP for high temperature sensing of oxygen. The value of the novel material is its dual potential to form hermetic seals with YTZP and also to conduct electrical charge.

**Keywords:** Fuels\Energy\Petrochemical, High Temperature, Microwave, Specialty Gas Analysis

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

**Methodology Code:** Electrochemistry
This research is focused on investigating a unique high surface area copper catalyst for the electroreduction of carbon dioxide (CO2). The nanoporous Cu catalysts were made by dealloying a Cu alloy in strong base to form a continuous nanoporous structure. Our BET data shows surface area of 10-40 m²/g depending on the alloy composition, pouring temperature, concentration of base, and etching time of the material. The nanoporous copper is assembled in an in-house designed electrochemical flow cell for CO2 electroreduction studies. Products were detected via gas chromatography couple with mass spectrometry and a thermal conductivity detector, ion chromatography, and nuclear magnetic resonance spectroscopy. Preliminary work has shown promise in nanoporous copper for its unique product distribution compared to conventional Cu foils and Cu single crystals. We will also report the product distribution as a function of the composition and morphology of the nanoporous catalysts. Electrochemical studies were performed using 0.1M KHCO3 electrolyte, Ag/AgCl (3M) reference electrodes, a Nafion® divider, and a DSA® counter electrode. The cell design allows electrolyte to continuously flow across the catalyst. A separate flow is used on the counter electrode side.
Hydraulic fracking is the use of fluid and material to create or restore small fractures in a formation in order to stimulate production from new and existing oil and gas wells. The process includes steps to protect water supplies. To ensure that neither the fluid that will eventually be pumped through the well, nor the oil (or gas) that will eventually be collected enters the water supply, steel surface or intermediate casings are inserted into the well to depths of between 1K and 4K ft. Water and sand make up 98-99% of the fluid used in hydraulic fracturing. In addition, chemical additives such as acid solutions, scale inhibitors, stabilizing agents, corrosion inhibitors, friction reducing agents, gelling agents, etc. are used. In this poster presentation, chemical analysis methodologies using ion analytical instruments will be discussed.
Laser Induced breakdown spectroscopy (LIBS) has been widely used in the analysis of solids and liquids but has had relatively less attention in the analysis of gaseous samples. LIBS spectra of helium, argon and nitrogen were acquired using samples of pure gases at low pressures and the spectra from the three samples showed continuum contributions which differ from one gas to the other. To better understand LIBS of gaseous samples, we have performed a study of Laser induced plasma in three gases i.e. Ar, He, N2 and their mixtures. The evolution of plasma intensity and electron density, with time was studied at different laser pulse energies. The plasma Intensity showed a decreasing pattern with increasing gate delay, while the electron density was inferred from stark broadening of an observed emission line. The spectrum emitted from the laser induced plasma can be simulated using the assumed plasma composition, plasma temperature and electron density and the spectroscopic constants of the emission lines from the NIST Atomic Spectra Database. To investigate the effect of plasma background, gas composition, spectral interference, etc to the observed LIBS spectra, the simulated LIBS spectra of the gaseous samples were compared with experimental spectra. This study will help to improve the understanding of laser induced plasma for more accurate LIBS spectral simulation which is important for direct gas analysis with LIBS using a LIBS database of simulated LIBS spectra.

Keywords: Laser, Plasma, Spectroscopy
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Combustion Ion Chromatography (CIC) is a powerful solution for the problem of identification and quantification of individual halogens, as well as total sulfur, in intractable samples. Difficult to process samples such as coals, crude oils, ores, solders, petrochemical gases, and polymer products such as printed circuit boards, to name a few, all can easily be analyzed by combustion in an oxidizing atmosphere at high temperature. CIC detection limits are not as low as those achieved by the use of ion chromatography on ordinary water samples, however. Sample size in CIC is generally limited to about 100 mg. Additionally, the sparging process used to trap the product gas containing the analytes is inherently dilutive. Techniques to improve detection limits and reproducibility will be discussed, principally those relating to inline pre-column concentration. Inline sample processing to remove interfering matrix constituents will also be considered.
Since 1950s, off-line combustion techniques like Wickbold apparatus and Schoeninger flask for petrochemicals and petroleum products is popular. However, these techniques are very labor intensive and not cost effective in fast pace laboratories in modern times. The fully automated combustion ion chromatography (CIC) system presented here combines a highly efficient combustion system with the separation power of ion chromatography (IC). CIC allows for the simultaneous speciation of halides (F, Cl, Br and I) and sulfur compounds (as sulfate) from sub-ppm to per cent levels in any sample matrix. Various applications for real world samples like Diesel fuel, gasoline, petroleum products, polymers will be presented.

Keywords: Elemental Analysis, Energy, Fuels\Energy\Petrochemical, Ion Chromatography
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Liquid Chromatography
Since 1946 when William Stephens proposed the concept of using electric fields to accelerate and eject ions in a field-free region to induce separation according to differences in flight times, the time-of-flight (TOF) mass analyzer has evolved in the areas of acquisition rate, mass accuracy, resolution, and sensitivity. Advances in concepts, electronics and computational processing capabilities allowed a new technology, the folded flight path TOF with high resolution, high accuracy and high acquisition rate analysis. The coupling of multi reflecting TOF with gas chromatography (GC-HRTMS) and liquid chromatography (LC-HRTMS) permits high performance analysis of complex mixtures providing good platforms for petroleomic studies. Using a petroleomic software package, we process data from several instrument platforms including GC-HRTMS using electron ionization (EI) and chemical ionization (CI), and LC-HRTMS using electrospray (ESI) and atmospheric pressure chemical ionization (APCI). The integration of these analytical platforms allowed us to investigate all possible elemental compositions for a broad characterization of petroleum products.

Keywords: Electrospray, GC-MS, Petroleum, Time of Flight MS
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Mass Spectrometry
Scale formation in boilers degrades their performance, and is mitigated by additives in the boiler water. Polyacrylic acid (PAA), a widely used anti-scalant additive, has both scale inhibition and dispersion properties. It is typically used at rates of 1 – 1000 µg/L, depending on the load of metals in the boiler water. The amount of PAA remaining needs to be measured for both process control and for wastewater discharge. Since PAA is the only macromolecule normally present in boiler water, it can easily be resolved using size-exclusion chromatography. Unfortunately, PAA only has a weak UV chromophore so UV absorbance detection is limited to more concentrated samples. Charged aerosol detection, a mass-sensitive approach capable of measuring any non-volatile and many semi-volatile species, shows excellent sensitivity, a wide dynamic range and does not require a chromophore for analyte detection.

Keywords: Coal, Energy, HPLC Detection, Water
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Liquid Chromatography
Natural gas is formed by a mixture of gaseous, main component is methane (CH₄), but also contains heavier HC: ethane (C₂H₆), propane (C₃H₈), butanes (C₄H₁₀), pentanes (C₅H₁₂). Percentages of CO₂ and gases such as nitrogen and oxygen are present. The conventional measuring systems are gas chromatographs with TCD detector. A complete analysis requires at least 3 analytical columns:
1. He, H₂, O₂, N₂, CH₄, CO
2. C₁-C₃, CO₂
3. C₄-C₁₀

The main goal of this work is to have a single MEMS column to share CO₂ – N₂ – CH₄ and C₂-C₆ in only one analytical run on chip silicon technology. The instrument need to work continuous in a remote location with a large range of ambient condition between -10 to +50 °C. A full MEMS based micro-GC, with his small-sized, is the best solution to control temperature of the “oven” easily, with a heating and Peltier plate; the temperature for this analytical method is between 10 and 15 °C, that means we have to heat in winter and cold down in summer time. With same analytical performance would therefore be the ideal solution for an effective continuous sampling of the above-mentioned parameters, for quick and easy control analysis and calorific power calculation. A pre-series of a miniaturized instrument formed of micro processed components in silicon chips had been realized, able to allocate in reduced dimensions the injector, the column and the detector, and able to minimize energy, flows and gas consumption, are installed on Natural gas secondary distribution cabinet. The MEMS elements allow to separate and quantify the various components of the natural gas, including CO₂ and N₂ in a single run. An impressively short analysis time and better separation power than the ones obtained with the conventional instruments had been obtained for HC, CO₂ and N₂.

The control of temperature is very smart and precise.

**Keywords:** Fuels\Energy\Petrochemical, Gas Chromatography, Lab-on-a-Chip/Microfluidics, Nanotechnology

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

**Methodology Code:** Process Analytical Techniques
At a fundamental level, both charged aerosol detection and evaporative light scattering detection (ELSD), two universal aerosol-based LC detectors, share some similarities in that mobile phase exiting an LC column is first nebulized and then dried to form analyte particles. However, the mechanism by which these aerosol-based techniques measure analyte mass differs markedly and this has major impact on analytical performance. With charged aerosol detection, charged particles are measured by an electrometer generating a signal that exhibits a straightforward proportionality to particle size over a wide analyte mass range. For ELSD, particles are measured by a photomultiplier generating a signal proportional to particle size, but this relationship is much more complex, as the efficiency of light scattering varies dramatically with particle size. This results in complex, often sigmoidal response curves, with relatively limited dynamic range and often poor precision. Furthermore, efforts to extend the lower limits of ELSD detection typically involves increasing photomultiplier gain to levels that are easily saturated. Unlike charged aerosol detection, changes in ELSD gain uses non-contiguous signal attenuation of the photomultiplier. As each attenuation setting has its own unique response profile that results in different sensitivities, calibration curves and dynamic range, samples may have to be reanalyzed multiple times in order to quantify analytes occurring at different concentrations. In this poster the analytical performance of charged aerosol and light scattering detection are evaluated and include: sensitivity, dynamic range, inter-analyte response, linearity, reproducibility and the effects of mobile phase flow rate.

Keywords: Detector, HPLC Detection, Instrumentation, Light Scattering

Application Code: General Interest

Methodology Code: Liquid Chromatography
Fuels, Energy and Petrochemicals Analyses

A Smart Phone of Potentiometric Titration Has Now Arrived

Titration analysis has stepped up to the next level within the Petrochemical Industry, leaving a trail of redundant titrators behind!
Capacitance touch screen and Automatic Electrode Recognition Technology (AERT) are the principles of the PAT 940, breaking new ground in electrochemistry. The Petrochem Analysis Titrator from GR Scientific has a cutting-edge look, designed with the user in mind to offer simplicity and easy-operation without sacrificing accuracy and precision.

A compact controller module utilizes a ‘light touch and scratch resistant’ interface enabling the user to navigate easily through the firmware, faster than other titrators in the market place. Selecting a method could not be easier with the new advancements in AERT. Simply plug in an EChem® electrode and the controller module automatically lists which methods are associated with that electrode in an instant. Pre-programmed methods conforming to ASTM standards as well as the ability to edit and save, guarantees ease of use. A unique colour coding system provides visible association between workspace and media so you always know at what stage you are during your analysis.

Advancing the technology further, PAT 940 has multiple burette capability allowing up to 15 simultaneously connected devices which means the possibility of running TAN and TBN methods side by side is now a reality!

Keywords: Electrochemistry, Electrodes, Fuels\Energy\Petrochemical, Petrochemical
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Electrochemistry
Military jet fuel (JP-8) is very similar to commercial jet fuel (Jet A) except for the presence of three additives, FSII (Fuel System Icing Inhibitor), CI/LI (Corrosion Inhibitor/Lubricity Improver) and SDA (Anti-Static Additive), that are added to improve specific characteristics of JP-8. Of particular interest is the CI/LI additive; the most common active being a dimer of linoleic acid. This paper focuses on exploring the possibility of qualification and quantification of the active ingredient in the CI/LI additive by liquid chromatography-mass spectrometry.

Dilinoleic acid was separated using liquid chromatography using a C8 column and an isocratic mobile phase of methanol with 0.1 vol % acetic acid at a flow rate of 0.3 mL/min. The acetic acid was used to alter elution and improve peak shape. Each run took approximately 10 minutes to complete, with the dilinoleic acid peak eluting at approximately 1.2 minutes along with many other polar components of the jet fuel. Time of flight mass spectrometry with electrospray ionization (negative ion) was used to detect the dilinoleic acid. An EIC (extracted ion chromatogram) of m/z 561 was used to isolate the dilinoleic acid peak from the other components. A calibration curve (2 different days) for dilinoleic acid is shown in Figure 1. The linear calibration curve indicates that dilinoleic acid can be determined in a jet fuel matrix. The dilinoleic acid content of several samples of jet fuel (JP-8) was determined to examine the range of additive content expected. This method will allow the determination of CI/LI content in military jet fuel samples.

Keywords: Fuels\Energy\Petrochemical, Liquid Chromatography/Mass Spectroscopy
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Liquid Chromatography/Mass Spectrometry
**Session Title**: Fuels, Energy and Petrochemicals Analyses  
**Abstract Title**: Construction of a Novel Densitometer that Utilizes a Near-Infrared Laser System  
**Primary Author**: Satoru Tsuchikawa  
**Nagoya University**  
**Co-Author(s)**: Ryunosuke Kitamura  
**Date**: Monday, March 03, 2014 - Morning  
**Time**:  
**Room**: - Exposition Floor, Back of Aisles 1

**Abstract Text**

We have designed a novel densitometer that consists of a continuous wave near-infrared laser source and an avalanche photodiode module as the detector, which can rapidly and non-destructively measure the density of wood. The wood density of a small area (3.14 mm²) was continuously estimated using the intensity of the transmitted light with the aid of the modified Lambert-Beer law. By conducting a validity evaluation with statistical coefficients, it was shown that the constructed system is as accurate as a conventional x-ray densitometer. It was concluded that the constructed NIR device has high performance from the viewpoint of operability, measuring time and safety.

**Keywords**: Laser, Light Scattering, Near Infrared, Paper/Pulp  
**Application Code**: Other (Specify)  
**Methodology Code**: Near Infrared
Lurgi FBDB Gasification Technology is used for the production of synthesis gas from coal. The raw synthesis gas exiting the gasifier contains hydrocarbons and water both in gaseous phase. During the cooling, the hydrocarbons are condensed including the water vapor present thus forming a condensate liquid stream. From the condensate streams, Naphtha, tar and oil are recovered as co-products.

As the co-products are complex mixtures of condensable carbon-rich materials, a detailed study on the co-product’s composition and chemical structure will be of great advantage to its further processing and utilization.

In this work, to analyze the chemical composition of the co-products, GC/MS and comprehensive GC/MS were used to analyze the volatile fractions. An efficient method is developed for the simultaneous determination of aromatics, aliphatics and phenols present in the co-products. The analysis results shows that naphtha is characterized by a high aromatic content of benzene, toluene and ethylbenzene & xylene (BTEX), whereas oil and tar contain non-trivial mixtures of compounds with minor concentration and a limited number of chemical classes, where the focus of the study was on classes of compounds instead of individual ones. Moreover, since the structured chromatograms produced by comprehensive GC allow clear visualization of chemical classes, the similarities and differences between samples were also studied.

For the non-volatile fraction of the co-products, the use of Thin-layer chromatography to determine the mass distribution of the co-products was studied. The FT-ICR MS with different ionization methods was used to determinate the elemental composition, CcHhNnOoSs, and identify the compound classes.

In this work, the analysis results of the Lurgi’s FBDB Gasification co-products show the possibility as the important or unique source for some aromatic hydrogen components besides the fuel use of the co-products.
Measuring soot in engine oils is an important parameter of engine health. Thermogravimetric analysis (TGA) has been a traditional method for determining soot content in a used lubricant as described in ASTM method D5967. High TBN additive pack interferences along with time and cost considerations mean that Infrared spectroscopy methods per ASTM E2412 and/or ASTM D 7844 are used for analysis of routine samples. These methods employ a single point measurement technique and do not have any baseline correction factors, which can significantly affect the reproducibility of a measurement under different conditions and between different instruments. This presentation will describe an improved procedure using a 4 point calibration and a weighted baseline that is standard on the Fluidscan, a handheld infrared grating spectrometer, with examples from actual locomotive oil applications.

Keywords: Contamination, Fuels\Energy\Petrochemical, Infrared and Raman, Portable Instruments
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Portable Instruments
Determining kinematic viscosity with microliter amounts of Newtonian fluid is possible with a novel design based on the Hele-shaw viscosity technique. Solvent free, fixed temperature, ability to verify calibration in the field, ranging up to 700 Cst at 40°C, this methodology provides opportunities for field based viscosity measurement for performance fluids. An overview of the technology and case examples will be presented.
Fuels dilution by ASTM D 3524 (GC with headspace sampler) is the referee method for fuel dilution in oil laboratories. SAW sensor devices modeled on Henry’s law have been used by the US navy for many years for field analysis of fuel in lubricating oil, a requirement for shipboard prime movers. Recent improvements to the headspace design and electronics provide an improved method for detecting volatiles in oil, and may be calibrated to correlate with existing GC Methods. A review of the technology, recent improvements and results discussion will be provided.

Keywords: Fuels\Energy\Petrochemical, Headspace, Portable Instruments, Semi-Volatiles
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Portable Instruments
Early detection of abnormal wear debris particulate is essential to predictive maintenance of mining equipment. A new approach to machine condition monitoring is presented. Filter Particle Quantification (FPQ) is a technique to concentrate debris for elemental analysis. Particles greater than 4 micron are measured and elemental data is derived from the patch. This solvent free two step approach provides insight to machine conditions in a consistent, easy to use method that lends itself to mobile oil analysis tools that can be brought to equipment at the mine face. This presentation will describe the concept, examples of failure detection, and introduce an expeditionary fluid analysis tool that combines this novel approach to wear debris with lubricant condition measurements.

Keywords: Particle Size and Distribution, Portable Instruments, Sample Preparation, X-ray Fluorescence
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Portable Instruments
Recently new process technologies for heavy oil upgrading have been developed in order to convert heavy crude oils into more valuable, lighter crude oils. A very important characteristic of crude oils is the boiling point distribution, from which the increase in distillate yields can be calculated. True boiling point (TBP) physical distillation is a time-consuming procedure (a minimum of eight hours), and it would be preferable to use Simulated Distillation by gas chromatography, a method that requires a relatively short time (typically less than an hour for the complete analysis). Heavy crude oils of 12.7 and 13.7°API were hydrotreated in a pilot plant at 703 K and 56 bar, resulting in upgraded crude oils of 13.4 and 16.5°API. The TBP distillation curve of the heavy and upgraded crude oils was determined by the ASTM D2892-13 and ASTM D5236-13 Standard Test Methods and by the ASTM D7169 GC-Simulated Distillation Method. The total distillates yield (up to 811 K) for the 13.4°API upgraded crude oil by TBP was 57.80 vol. % vs. 57.50 vol. % by GC-Simulated Distillation; the yield for the 16.5°API upgraded crude oil was 65.95 vol. % by TBP and 66.40 vol. % by GC-SimDist. These results show that GC-Simulated Distillation reproduces TBP distillation data for heavy and upgraded crude oils within the reproducibility limits with a reduction of analysis time, and quantity of sample. Thus, GC-Simulated Distillation is a fast and reliable method for assessing the increase in valuable distillates yield in the upgrading of heavy crude oils.

Keywords: Fuels\Energy\Petrochemical, Gas Chromatography, High Temperature
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography
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 their molecular ions, this important subject is being ignored and represents an untapped opportunity.

The 5975-SMB GC-MS with Cold EI was used for the development of a new method of isomer distribution analysis for fuels and oils characterization. Cold EI is based on GC and MS interface with supersonic molecular beams (SMB) and on sample compounds ionization using a fly-through electron ionization ion source while they are vibrationally cold (hence the name Cold EI). GC-MS with cold EI enables the analysis of large oil and wax compounds, up to C74, via the use of short columns with high column flow rates. The system provides trustworthy and largely enhanced molecular ions and isomeric structurally important high mass fragments to all analytes for their improved identification. Isomer distribution analysis (IDA) is based on their GC separation while the Cold EI provides molecular ions to all hydrocarbons isomers. The greater is the isomer branching the earlier is its elution temperature. IDA was explored for improved fuel, oil and hydrocarbon mixtures characterization in various petrochemical mixtures as well as in waxes.

**Keywords:** Fuels\Energy\Petrochemical, Gas Chromatography/Mass Spectrometry, Hydrocarbons, Petroleum

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

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Jet fuel constitutes a very complex mixture of primarily hydrocarbons, with traces of nitrogen and oxygen compounds. Polar components are responsible for a significant fraction of the deposits formed when jet fuel is stored under high temperature conditions. Jet fuel constitutes a very complex mixture of primarily hydrocarbons, with traces of nitrogen and oxygen compounds. In order to understand the reactions that occur in jet fuel, the reactions of polar, nitrogen containing aromatic compounds (dimethyl aniline, ethyl aniline and diethyl aniline) were investigated in a jet fuel surrogate under conditions were a limited supply of oxygen was present. The rates and products of the reaction were determined using normal phase high performance liquid chromatography (isooctane/isopropanol mobile phase) and gas chromatography-mass spectrometry. A surrogate fuel comprised of n-dodecane, diethyl benzene, methyl isopropyl benzene and diethyl cyclohexane was chosen to simulate the paraffinic, aromatic and naphthenic components typically found in fuels. It was found that the anilines react rapidly to give colored solutions and several oxidation products appeared in the HPLC chromatogram. The products of the reactions were isolated by collecting fractions from the HPLC column and evaporating the solvent. The products of the reactions were identified by nuclear magnetic resonance spectroscopy.

Keywords: Fuels\Energy\Petrochemical, Gas Chromatography/Mass Spectrometry, HPLC, NMR
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Liquid Chromatography
Fluid Catalytic Cracking (FCC) and Hydrocracking are unit operations refineries employ to convert low-value, high molecular weight feedstocks into lighter, high-value products by cracking C-C bonds. The nitrogen content of crude petroleum is generally in the range of 0.1% - 0.9%, however some feedstocks may contain up to 2% nitrogen. Organic nitrogen compounds found in petroleum include indole, carbazole, pyridine, and quinolone.

Cracking organic nitrogen compounds liberates hydrogen cyanide, ammonia, and other nitrogen compounds. Sour wastewater is formed when hydrocarbons, H2S, and gas phase reaction products are transferred to a distillation column where steam is injected to reduce hydrocarbon partial pressure.

Sour wastewater contains emulsified oils, phenols, sulfides, mercaptans, ammonia, and cyanide. Many constituents of sour wastewater are known matrix interferences and complicate measurement of cyanide species. This poster will describe techniques for accurately measuring cyanide species in the presence of these interferences and present analytical data obtained on and sour wastewater samples.

Keywords: Environmental/Water, Fuels\Energy\Petrochemical, Ion Chromatography, Wet Chemical Methods
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Chemical Methods
Over the last years the Institute of Measurement Technology has developed a fast high-resolution mass spectrometer system for the quantification of hydrocarbon emission of automotive engines. In this harsh environment the problem of surface contamination of the ion source arises. The surface contamination leads to a reduced response time and background signals over measurement time. To eliminate these factors the result is a minimalistic design that especially reduces the surface of the ion source body. The ion source is built on the concept of standard EI ion sources and designed to be adapted to Varian 1200 and to Varian 320 mass spectrometers. In order to find an optimal setup and to prove the functionality simulation software has been used. Different geometric setups were simulated and based on the results lead to the final design of the ion source. The ion source has then been installed into a Varian 1200-MS. Measurements have been carried out that show the ability to compete with the standard Varian ion source with respect to sensitivity and signal to noise ratio by having less issues with surface contamination. Due to its simple design and contamination stability this ion source reduces maintenance effort to a minimum. The setup, functionality and simulation will be presented.

Keywords: Contamination, Hydrocarbons, Mass Spectrometry, Quadrupole MS
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Mass Spectrometry
The recent increase in U.S. natural gas production has been propelled by the extensive use of hydraulic fracturing (also known as fracking). This process extracts natural gas by drilling into bedrock (primarily shale) and then injecting fluid under high pressure causing cracks in bedrock, thereby releasing trapped gas, which can then be captured. While fracking provides financial benefits to both local and national economies, it has not been without controversy. Inadvertent spills or the dumping of fracking flowback (fracking solution that returns to the surface) into unlined collection ponds can contaminate ground water. One of the challenges with analyzing flowback water is the high levels of dissolved solids as salts that are leached from bedrock. Injection of undiluted high ionic strength solutions can exceed the capacity of columns used in ion chromatography (IC), resulting in poor chromatography, peak suppression, and inaccurate reporting. Additionally, high concentrations may exceed the linear range for a particular analyte. To prevent column overloading by samples such as fracking flowback water, dilutions are performed. To minimize errors and reduce labor costs associated with manual dilutions, this process has been automated using in-line conductivity measurement, which then triggers dilution by an autosampler. This poster describes the analysis of inorganic anions and organic acids in fracking flowback water using IC following in-line determination of sample conductivity and automated dilution.
Ion channels (ICs) are complexes of transmembrane proteins that regulate the passage of specific ions across biological membranes. ICs play vital roles in regulating cellular excitability, establishing a resting membrane potential and numerous other biological processes. Electrophysiological measurements have been widely used as a highly sensitive and label-free approach to investigate the kinetic behavior and ligand response of ICs, though more complex structural questions prove problematic using this approach. Recently, ICs have been used as transducers in a range of biochemical and chemical sensing applications. As more chemically diverse ICs are utilized for this purpose, there is a clear need to better understand the insertion and orientation of ICs into artificial membranes.

Here we describe the development and application of an imaging platform that couples confocal single molecule fluorescence microscopy with electrophysiological recordings of channel function to facilitate direct observation of IC orientation, composition and insertion into black lipid membranes (BLMs). BLMs were suspended across micro-fabricated apertures prepared from SU-8 substrates that were subsequently integrated into the imaging chamber. We focused on the inward rectifier K+ channel, Kir 6.2, and Kir 6.2-EGFP fusion proteins. Kir 6.2 forms an ATP-sensitive channel comprised of four Kir 6.2 monomers, though the mechanism of channel assembly, insertion and orientational control remain unknown. Single molecule microscopy coupled with single channel electrophysiological recordings system allowed for the monitoring of Kir 6.2 EGFP insertion into BLMs, and will facilitate the determination of IC orientation and composition using highly luminescent antibody conjugates.

Keywords: Bioanalytical, Imaging, Lipids, Protein
Application Code: Bioanalytical
Methodology Code: Microscopy
Claudins are tight junction proteins that play an important role in regulating paracellular ion transport. Claudins interact to perform vital cellular functions. For example, claudin16 and 19 have been observed to interact with each other and confer tight junction with cation selectivity which is critical to renal reabsorption. However, localized study of claudin16 and 19 interactions on a single cell scale is still lacking. Scanning ion conductance microscopy (SICM) can be used to investigate biological samples, such as cell monolayer. Through modification of the conventional SICM configuration, a five-electrode potentiometric-SICM (P-SICM) has been applied to measure the local conductance at selected cell junction. Experimental results showed that we were able to differentiate paracellular and transcellular pathways and record claudin channel ion selectivity. Through selective labeling of claudin 16 and 19 and coexpression of the two claudins in one cell, P-SICM can be utilized to locate cell junctions and measure the claudin interactions.
Comparing Flow Cytometry, Fluorometry, and Confocal Microscopy Methods for Determining the Phagocytic Ability of Macrophages Pre-Exposed to Gold and Silica Nanoparticles

It has been previously demonstrated that systemic administration of silica and gold nanoparticles (NPs) leads to particle accumulation in macrophages of the reticuloendothelial system. The impact of this bioaccumulation on macrophage function, however, is not fully understood. This study evaluates the utility of three phagocytosis assays performed with flow cytometry, fluorometry, and confocal microscopy. RAW264.7 cells and primary cells isolated from the bone marrow of Balb/C mice were pre-exposed to non-toxic concentrations of 10 nm silica NPs, 10 nm gold NPs, or a series of positive and negative controls. Cells were then exposed to FITC-E.coli. Cells were analyzed by flow cytometry using a FacsAria III and DIVA software or a Tecan Safire fluorescent plate reader. For confocal analysis, cells were analyzed on Olympus FV1000. Post image analysis was performed using ImageJ to determine the percentage of cells that were able to phagocytose bacteria.

Results indicated that plate reader measurements did not generate significant signal over background values. Silica NPs did not interfere with any of the assays used, while gold NPs showed significant interference in the form of quenching. Confocal microscopy analysis proved critical in providing semi-quantitative data linking the observed fluorescent decrease with a decrease in phagocytosis. By combining the information from all assays, it was determined that phagocytosis was reduced by ~ 50% in cell populations pre-treated with NPs. Confocal microscopy was therefore determined to be a valuable supporting method to determine phagocytosis ability. This method has also been modified to analyze surface marker activation and viability, indicating that the technique may be a versatile platform for future studies. This project was supported in part by an appointment to the Research Participation Program at the Center for Drug Evaluation and Research administered by the Oak Ridge Institute for Science and Education.
Microscopy

Tissue engineering that involves matrix-specified differentiation of stem cells provides new approaches for tissue regeneration. In this study, we used electrospinning technique to fabricate well-aligned collagen type I fibers mimicking the native extracellular matrix (ECM), and examined the morphology, growth rate, and neural differentiation of human decidua parietalis placental stem cells (hdpPSCs). To enhance mechanical properties of e-spun fibers, we incorporated spider silk proteins into collagen. Results from mechanical tests and AFM force measurements showed that as silk protein content increased, both elasticity and dissolution resistibility of e-spun fibers increased significantly. The collagen-silk fiber alignment induced the cell polarization and promoted long filaments development. The immunostaining results revealed that hdpPSCs had greater differentiation abilities to neural cells when cultured on composite e-spun fibers with higher collagen to silk ratios. We also observed the variations in cell shape and dimension with fiber contents, likely caused by changes in cell adhesion. In order to evaluate adhesive strengths between cells and collagen-silk composite e-spun matrices, we applied an AFM affinity measurement method to quantify the cell adhesion forces between a cell and each type of e-spun biomaterials. This method, along with a trypsin de-adhesion assay, showed that e-spun collagen-silk composite matrices with low silk content (15%, 30%) can provide more favorable substrates for hdpPSC attachment, proliferation and differentiation. To unravel the cell-matrix interaction, further efforts will be made to explore the role of integrin, the collagen distribution within an e-spun fiber, and potential structural changes of collagen in e-spun collagen-silk fibers. Overall, collagen-silk fiber alignment provides the guidance for neuronal cell development, demonstrating the potential of making bio-circuit for future applications in nano-bio-devices.

Keywords: Materials Characterization, Materials Science, Microscopy, Nanotechnology
Application Code: Materials Science
Methodology Code: Microscopy
Gold nanoparticles (AuNPs) have attracted a great deal of attention because of their unique properties and great variety of applications which range from microelectromechanical systems to biosensor devices. To optimize and extend the scope of AuNP applications, recent work has focused on controlling the assembly of AuNPs into one-, two- or three-dimensional structures. In particular, 1-D AuNP assemblies have received considerable attention because gold nanochains have similar optical properties as gold nanorods and nanowires such as the appearance of a new longitudinal plasmon band. Chainlike AuNP structures have been induced via use of surfactant, molecular linker, DNA duplex and polymer additives among others. However, in most of these instances, the formed AuNP nanochains are unstable and randomly connected together forming extensive branched networks which are similar to AuNP aggregates. In this work, we report a simple means by which to assemble AuNPs in one-dimensional chains without branches or aggregates by the use of a zwitterionic surfactant, e.g., 3-(decyldimethylammonio)-propyl sulfate [C\text{sub}10[/sub]APSO[sub]4[/sub]]. Through the cloud point extraction method, we demonstrate control of the assembly of AuNPs into chainlike structures as well as extracting and concentrating the assembled nanochain product in one step. The C[sub]10[/sub]APS\text{sub}4[/sub] surfactant plays three roles in this process; i.e., as an adhesive agent, capping agent and extracting agent. The assembled gold nanochains have an average particle size of 100 nm, exhibit unique optical properties and considerable stability. The length of gold nanochains can be controlled and tuned by merely changing the centrifugal force for the centrifugation step. Due to their unique properties and robustness, these gold nanochains may be potentially useful in detection systems, electronic applications, etc.

Keywords: Extraction, Nanotechnology, Sample Preparation
Application Code: Nanotechnology
Methodology Code: Chemical Methods
During recent centuries, significant attention has been paid for the development of sensorics especially there is a growing interest in the field of development of analytical methods in biotechnology for use in medical, environmental and food analysis. Among these analytical methods, biosensors have been selected as the most convenient systems for the analysis of biologically active compounds in complex samples as a result of their sufficient selectivity, high sensitivity, simple applicability, although they are not expensive and suitable for mass production [1]. For the development of biosensing platforms through the association of a successful immobilization of biorecognition molecules and an efficient signal transduction, nanoscale materials have been offered because of their excellent prospects [2-4]. In this case, carbon nanostructures have been largely used due to their exceptional structural, electronic and mechanical properties that make possible the development of electrodes with minimal fouling and fast electron transfer, mainly due to the presence of edge-plane like sites located at the end and in the defect areas of the tubules [5]. This study was one of the application of carbon nanostructures for the design of biosensors for the enhancement the sensitivity in the electrochemical determination of biomolecules. As a result of this study it was reported that proposed surfaces generated particular electrochemical responses as much as the development of systems for applications in analytical and biomedical sciences.

Keywords: Bioanalytical, Electrochemistry
Application Code: Nanotechnology
Methodology Code: Electrochemistry
Carbon nanodots (C-dots) have attracted enormous interest for their unique photoluminescence properties, low cytotoxicity, tunable surface chemistry, and high photostability. These properties have made them suitable for a wide range of applications. C-dots are typically separated by lower resolution separation means such as centrifugation, dialysis, and filtration. However, after these separation procedures, C-dots can still exist as a complex mixture (e.g., different size and charge). In this study, we used a bottom up approach to synthesize C-dots by means of the decomposition of citric acid. After centrifugation, filtration, and dialysis, the C-dots were separated by means size exclusion chromatography (SEC). Several separation conditions were studied while optimizing the SEC method. We will present in detail the separation conditions used to separate the synthesized C-dots. The C-dots mixture showed several chromatographic peaks, which were collected for further characterization by TEM, FTIR, and XPS. The photoluminescent properties of the mixture and each fraction were investigated and will also be presented.

Keywords: HPLC, Liquid Chromatography, Luminescence, Separation Sciences
Application Code: Nanotechnology
Methodology Code: Separation Sciences
Nanomaterials (NMs) have been gaining popularity in the past decade due to various useful properties of NMs compared to the bulk material. Due to these useful properties, various NMs have been synthesized on an industrial scale, and have found usage in the biomedical sector. However, one of the biggest shortcomings of NM synthesis, however, has been its high variability batch-to-batch. Even small variations in NM synthesis could result in differences in the interactions of these NMs with various biomolecules, which could lead to unintended side effects and potential cytotoxicity if introduced into the body. These changes may not be easily noticed by standard characterization methods such as transition electron microscopy, dynamic light scattering and zeta potential.

Fluorescamine is a non-fluorescent molecule that fluoresces upon binding to primary amines. This makes fluorescamine useful for the detection of protein-NM interactions for several reasons. Firstly, the reaction is rapid, being complete in less than five minutes. Also, proteins will partially unfold upon binding to NMs. This will expose additional amines on the protein's surface to fluorescamine, resulting in a net fluorescence increase for NM-protein interactions. These properties make the fluorescamine assay well-suited for high-throughput microplate analysis.

Various parameters (salt concentration and pH of the running buffer, concentration of fluorescamine added, and assay volume/time) were optimized for this assay. Afterwards, the assay was applied to interactions of model proteins with various nanomaterials (silica, latex, polystyrene). What was found was that the proteins capable of binding exhibited increased fluorescence compared to non-binding examples. In addition, an assay was done using proteins of varying size, charge and hydrophobicity, to determine if fluorescamine based methods can be used to determine batch-to-batch nanomaterial reproducibility in a rapid, simple manner.

Keywords: Bioanalytical, Fluorescence, Nanotechnology, Protein
Application Code: Nanotechnology
Methodology Code: Sensors
Nanotechnology can be used to make inexpensive plastic parts with functional surfaces. The plastic parts can be molded using a standard injection molding process. The nanostructures are directly transferred from the surface of the molding tool to the surface of the molded plastic part during the molding process. The main advantage with this method is that surface treatments and chemical additives are avoided, which minimizes health risks and simplifies recycling. Another advantage is that the unique technology enables nanostructuring of free form molded parts.

The functional surfaces can have many different properties including anti-reflective, self-cleaning, anti-stiction or color effects. In this work we focus on the superhydrophobic and self-cleaning properties.

Keywords: Materials Science, Method Development, Nanotechnology, Polymers & Plastics
Application Code: Nanotechnology
Methodology Code: Other (Specify)
A general, environmental-friendly in situ method for the fabrication of reduced graphene oxide (RGO)/metal (oxide) (e.g. RGO/Au, RGO/Cu2O, and RGO/Ag) nanocomposites using glucose as the stabilizer and reducing agent were developed. The RGO/metal (oxide) nanocomposites were characterized by STEM, FE-SEM, EDS, UV-Vis absorption spectroscopy, XRD, FT-IR and Raman spectroscopy. The reducing agent, glucose, not only reduces the GO to form RGO, but also reduces the metal precursors to form metal (oxide) nanoparticles on the surface of RGO. Moreover, the RGO/metal (oxide) nanocomposites are stabilized by glucose on the surface of RGO. To demonstrate the utility of these materials for electrochemical sensing, RGO/Au nanocomposite was used for the simultaneous detection of L-ascorbic acid (L-AA), dopamine (DA) and uric acid in natural matrixes with detection limit of 0.1 mM, 1 µM and 5 µM, respectively.

Keywords: Biosensors, Electrochemistry, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Electrochemistry
A facile bottom-up method for the synthesis of highly fluorescent graphene quantum dots (GQDs) has been developed using a one-step pyrolysis of a natural amino acid, L-glutamic acid, with the assistance of a simple heating mantle device. The developed GQDs showed strong blue, green and red luminescence under the irradiation of ultra-violet, blue and green light, respectively. Moreover, the GQDs emitted near-infrared (NIR) fluorescence in the range of 800-850 nm with the excitation-dependent manner. This NIR fluorescence has a large Stokes shift of 455 nm, providing significant advantage for sensitive determination and imaging of biological targets. The fluorescence properties of the GQDs, such as quantum yields, fluorescence life time, and photostability, were measured and the fluorescence quantum yield was as high as 54.5 %. The morphology and composites of the GQDs were characterized using TEM, SEM, EDS, and FT-IR. The feasibility of using the GQDs as a fluorescent biomarker was investigated through in vitro and in vivo fluorescence imaging. The results showed that the GQDs could be a promising candidate for bioimaging. Most importantly, compared to the traditional quantum dots (QDs), the GQDs is chemically inert. Thus, the potential toxicity of the intrinsic heavy metal in the traditional QDs would not be a concern for GQDs. In addition, the GQDs possessed an intrinsic peroxidase-like catalytic activity that was similar to the graphene sheets and carbon nanotubes. Coupled with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), the GQDs can be used for the sensitive detection of hydrogen peroxide with a limit of detection of 20 [micro]M.

Keywords: Amino Acids, Biomedical, Fluorescence, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Fluorescence/Luminescence
It has been demonstrated that microfluidic systems allow integration of sampling, reagent mixing, and rapid electrophoretic analysis. They have also proven useful for culturing cells wherein control over the environment allows novel and automated experiments. In this work we have devised such a system for studying islets. Pancreatic β-cell—the only cell secreting insulin in the human body—is the one of the major components of islets of Langerhans. It is well-known that islets have insulin oscillation with 3-5 min periods; some evidence also suggests a secondary ultradian (50-120 min) or even circadian rhythm. Deficiencies in such rhythms have been observed in Type 2 diabetes (T2D) patients. To continuously monitor single islet insulin secretion patterns for 24 h would be a helpful approach to understand these rhythms and T2D. We have developed a microchip based electrophoresis assay allowing long term monitoring of insulin secretion from islets. The system incorporates two independent syringe pumps loaded with low and high concentration glucose. Flow rates of both are controlled by a home-made LabView program. By mixing two syringe pumps' contents at different flow rates through a Tee fabricated on the electrophoresis chip, 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, thus allowing continuous monitoring of insulin under well-controlled conditions. This “islet workstation” has been used to study single islets oscillation patterns during 24 h time period.

Keywords: Bioanalytical, Capillary Electrophoresis, Immunoassay, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Semiconductor nanocrystals (SCNCs) can be considered as charge state definable redox centers, which are capable of donating or accepting electrons. The inter-nanocrystals charge transport property is crucial to enhance the performance of solid-state devices. The delocalization of the exciton, which is a SCNC bound electron-hole pair, is important to control the charge transport properties. The exciton delocalization over multiple SCNCs can be represented as electronic coupling. The electronic coupling can influence many photophysical properties including the band gap of SCNCs. Herein we show that ultra-small CdSe nanocrystals undergo strong electronic coupling, which can be controlled by modulating the thickness of “solvent-like” ligand-coating, poly(ethylene glycol) thiolate (PEG-thiolate). Various chain length PEG-thiolate-protected 1.6 nm diameter CdSe nanocrystals were synthesized at room temperature to control the exciton delocalization. It was found that as the glycol chain length increases, the lowest energy absorbance peak moved to shorter wavelength. This suggests that inter-nanocrystal electronic coupling decreases. The strong exciton delocalization between CdSe nanocrystals results in up to a 130 meV decrease in SCNC core energy. The ex-situ ligand exchange reaction on octylamine-coated 1.6 nm CdSe nanocrystals with PEG-thiolates was also performed, which suggests that exciton delocalization between multiple SCNCs can be restored by removing the insulating ligand, octylamine. The mass spectrometry studies have confirmed that during ligand exchange reaction, the core diameter of the CdSe nanocrystals was not compromised.
Bacteria comprise the highest biodiversity on Earth, and studies have revealed that there are still thousands, if not millions, of unstudied species. Discovery of new antibiotics has plummeted and there is a need to find new drugs to target diseases. Studying and understanding new microorganisms can address the underlying factors of many diseases, lead to new drug therapies, and help discover the missing pieces of microbial world. There is no automated methodology in literature to capture microorganisms. Therefore, we designed polymer (PDMS) microfluidic devices with constrictions that are tailored to the morphology of bacteria to isolate distinct species from heterogeneous samples.

We fabricated devices that have multiple sub-micron constrictions connecting food channels to one large entrance channel. Once the heterogeneous sample is introduced into the large channel, bacteria will move toward fresh growth media located in the food channels and get trapped in size-specific constrictions. Different species can be captured in each constriction and separated from the sample. Devices were fabricated by first creating a master mold using electron-beam and optical lithography and then using soft lithography to produce patterned PDMS chips. In this study, cyan fluorescent protein labeled Pseudomonas aeruginosa and m-cherry labeled Escherichia coli were mixed and loaded into a device that was filled with lysogeny broth and then incubated at 37 degrees Celsius for 24 hours. Fluorescent images confirmed successful separation and isolation of [i]P. aeruginosa[/i] from [i]E. coli[/i]. The constrictions were too narrow for [i]E. coli[/i], but large enough for [i]P. aeruginosa[/i] to enter and divide. This method can be easily implemented [i]in-situ[/i] for rapid separation of bacteria.

This project was supported by NSF Award #1125535.
The placement of electrodes within nanochannels realizes benefits related to enhanced mass transport – greater current density, conversion efficiency, and response time. Flow in nanochannels is preferentially driven via electrokinetic flow, because of the onerously high pressure needed to generate flow through nanochannels. Currently, the application of electrochemistry in electrokinetic flow is limited to bipolar electrodes with indirect current measurement. At sufficiently high overpotentials, arrays of embedded annular nanoband electrodes induce electroosmotic flow, thereby increasing the limiting current and conversion efficiency. The utilization of a second working electrode further increases the electroosmotic flow. This represents an overall 30x increase from a similar structure with no convective flow. To further extend system control, an isolated potentiostat was developed in order to control of the electrode potential independent from the solution potential. This allows for the placement of a working and quasi-reference electrode within channels regardless of field strength or local potential.
When insonified by narrowband unidirectional ultrasound, small particles in suspension are driven into motion away from the transducer. The resulting particle velocities can be measured by observing Doppler shifts in the backscattered ultrasonic signal. Contrary to some theoretical expectations, the velocities of particles smaller than about 100 nm so excited vary inversely with particle size, i.e., the smallest particles attain the greatest velocities. To establish a quantitative relationship between size and velocity, identically prepared samples of various size ranges of polystyrene and colloidal silica (Ludox) particles were imaged by transmission electron microscopy (TEM) to measure particle size distributions (PSD) and their Doppler shifted backscatter spectra were measured by the USPD method. To compare these measurements, the TEM-determined PSD was converted to an "expected" backscattered spectrum by multiplication of the relative number in each histogram bin by its particle diameter to the sixth power based on the Rayleigh-scattering dependence of scattering intensity on particle size. Comparison of features in the USPD-measured and TEM-expected spectra were used to correlate particle size and Doppler-shifted velocity to construct a calibration curve. Calibration curves for the two types of particles studied were similar, thereby suggesting that the calibration curves for a variety of materials may be comparable. This technique is being investigated as a simple and inexpensive means for measurement of PSD of nanoparticles. Because a single transducer can be used to both insonify the sample and to acquire the resulting backscatter signal, the USPD method can be employed in a batch-oriented free standing instrument or can be incorporated into existing equipment with an in situ transducer. The method is nondestructive and, in contrast to light scattering methods, can be used with suspensions that are not transparent.
Emerging materials—such as graphene, carbon nanotubes and SiC—require comprehensive characterisation before they can be used in future applications, such as batteries, inverters, and light emitters. Raman spectroscopy is a well-established technique for investigating strain, electronic properties and homogeneity in these materials, with sub-micrometer spatial resolution. However, with the upscaling required to move towards commercial production, it becomes challenging to analyse sufficiently large amounts of material in the required detail.

In this work, we demonstrate advancements in hardware and software that enable these materials to be characterised on the macroscopic scale, in practical time scales, whilst still maintaining sub-micrometer resolution. Large datasets can be collected quickly, enabling the application of statistical techniques to easily detect non-uniformity.

These improvements are also applicable to tip enhanced Raman spectroscopy (TERS). This technique combines Raman and AFM, and uses a plasmonic tip to produce chemical information with a resolution better than the optical diffraction limit. Here we report on a semi-automated method that finds the active region of the TERS tip by Raman mapping in three dimensions, saving significant time and making measurements on weak TERS scatterers possible. This method can also be used to optimise the enhancement signal, reducing TERS measurement times.

A comprehensive scan of a potential TERS tip identifies if the tip is TERS active, allowing inactive tips to be discarded with certainty. This removes some of the “witchcraft” from TERS experiments.

Keywords: Nanotechnology, Raman, Semiconductor, Vibrational Spectroscopy
Application Code: Nanotechnology
Methodology Code: Vibrational Spectroscopy
Nanoparticles have a wide range of use in different fields of industry, medicine and basic science. Many techniques are used for synthesizing silver nanoparticles, such as chemical reduction of metal salt. However, because of toxic effect of nanoparticles, various techniques are developed. Recent studies show that using biological synthesis of metal nanoparticles is more less toxicity and safe for environmental condition. The biological method for the synthesis of nanoparticles employs use of biological agents like bacteria, fungi, yeast and plants.

The aim of study were production of silver nanoparticles using [i]Triticum durum[/i] extract then the antimicrobial activity of this extraction has been investigated.

Green synthesized AgNPs were characterized by UV-visible spectroscopy, Electron microscopy technics, Raman spectroscopy analyses etc. Nitrate reductase enzyme activity analyzed during synthesis of silver nanoparticles.

Antimicrobial activities showed against Gram negative; [i]E.coli DH5[\textit{\textregistered}], Pseudomonas aeruginosa PAO-1, Pseudomonas aeruginosa PA14, Klebsiella pneumonia, Salmonella typhmurium 14074, Acinetobacter baumanii, Agrobacterium tumafacis A136[/i] and Gram positive bacteria; [i]Staphylococcus aureus, Bacillus subtilis 6633, Bacillus megaterium, Staphylococcus epidermidis[/i], and pathogenic yeast; [i]Candida keyfr, Candida tropicalis, Candida krusei, Candida parapsilosis, Candida albicans 10231[/i].

Keywords: Agricultural, Biotechnology, Drug Discovery, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Microscopy
Direct detection of low concentrations of nucleic acid biomarkers of disease without the use of target amplification technologies like PCR (Polymerase Chain Reaction) or NASBA (Nucleic Acid Sequence Base Amplification) is an important analytical objective.

In this contribution we report on a fully integrated microfluidic device (Figure 1) that incorporates an electrochemical detection strategy capable of detecting sub-femto molar concentrations of DNA [1]. The detection electrode is modified with capture strand nucleic acids that are complementary to part of the target. Once the target has been hybridised to the capture strands, regioselectively functionalized electrocatalytic platinum nanoparticles [2-5] that are labelled with probe DNA that is complementary to the non-hybridised section of the target are introduced. These platinum nanoparticles generated a significant electrocatalytic current for hydrogen peroxide reduction [6-7] whose magnitude depends linearly on the target concentration. The performance of the system for the detection of Staphylococcus aureus DNA without amplification is described (Figure 2).

The simplicity and ease of use of this biosensor platform makes it an amenable and versatile platform for automation and fabrication, making polymeric substrates more attractive for point-of-care diagnostics.

References

Keywords: Biosensors, Electrochemistry, Lab-on-a-Chip/Microfluidics, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
The availability of portable instrumentation for characterizing surface topography on the micro- and nanometer scale is very limited. Particularly the handling of curved surfaces, both concave and convex, is complicated or not possible on current instrumentation. However, the currently growing use of injection moulding of polymer parts featuring nanostructured surfaces, requires an instrument that can characterize these structures to ensure replication-confidence between master structure and replicated polymer parts.

This project concerns the development of a metrological traceable quality control method with a portable instrument that can be used in a production environment, and topographically characterize nanometer-scale surface structures on both flat and curved surfaces. To facilitate the commercialization of injection moulded polymer parts featuring nanostructures, it is pivotal that the instrument can characterize and validate the micro- and nanoscale topography directly in the production facility, as the interruptive time delay induced from shipping to an external facility is not compatible with present large-scale production routines. Satisfactory characterization of nanostructured curved surfaces in vibration prone production facilities has not previously been reported in the literature, and therefore has great novelty potential.

Keywords: Characterization, Microscopy, Nanotechnology, Portable Instruments

Application Code: Nanotechnology

Methodology Code: Surface Analysis/Imaging
We have recently realized an electron probe microanalyzer (EPMA) \([1,2]\) and cathodoluminescence (CL) spectrometer \([3]\) with a palm-top size chamber including the electron source and the sample stage using a pyroelectric crystal as the electron source. In the present study, we carried out elemental analysis and mapping in micro-scale regions using the portable EPMA and CL spectrometer. As for the portable EPMA (elemental analysis), electron beam was focused on a sample by applying an electrical field to a metal needle tip set on the pyroelectric crystal. The spot size of the focused electron beam was 300 \(\mu\)m. We succeeded in elemental analysis in a few hundred micrometers region using the focused electron beam. The portable CL spectrometer can capture luminescence of rare earth elements (CL image) using a CMOS camera. Applying this property, the portable CL spectrometer can perform an elemental mapping of particles with the diameter of tens of micrometer using a digital single-lens reflex camera.

Nanoscale Chemical Imaging of Membrane Receptors by Tip Enhanced Raman Spectroscopy

Studying biological samples with chemical specificity on the nanometer scale poses challenges for optical microscopy. Technical difficulties involved in such pursuit include breaking the diffraction limit and generating sufficient chemical specific signals. Tip Enhanced Raman spectroscopy (TERS) is one technique that addresses these difficulties and is capable of imaging biologically relevant samples such as proteins and model lipids. Despite this progress, imaging specific molecules in intact cells remains challenging. We have developed a targeted TERS approach that combines TERS microscopy with targeted delivery of gold nanoparticles. Dark field microscopy of the nanoparticles bound to cells identifies the region of interest (ROI) on the cell. Coupling between the TERS tip and the nanoparticle increases the sensitivity of the TERS measurement. By employing a ligand conjugated Au nanoparticle (GNP) probe, we selectively target a specific membrane receptor and create an aggregated nanostructure in situ when the Au TERS tip scans over an anchored probe. Nano-probes with different functionality and specificity are explored to demonstrate the targeting capability and imaging efficacy of this method.

Keywords: BIOanalytical, Nanotechnology, Spectroscopy, Surface Enhanced Raman
Application Code: Nanotechnology
Methodology Code: Surface Analysis/Imaging
Structured surfaces can be used as substrates for surface assisted laser desorption/ionization mass spectrometry (SALDI-MS) to improve the performance. Although it has been proposed that the laser energy absorbed by SALDI substrate is transferred to the analytes and make the analyte vaporized and ionized, the effect of the absorbed laser energy and its dissipation and/or relaxation has rarely been reported due to the random morphologies of the SALDI substrate and the complexity of the process. For example, spot-to-spot variations in SALDI performance are inevitably caused by the random distribution of particles or structures.

In this work, the antireflective Si nanocone arrays are fabricated and used as SALDI substrates for small molecules analysis. The role of the absorbed laser energy and its distribution in the laser desorption/ionization process has been investigated. The absorbed laser energy can be channeled completely into the desorption/ionization of analytes by optimizing the surface features such as height and period. The optimized Si nanocone array exhibits the excellent desorption/ionization performance for detecting peptide, amino acid, drug molecule, and carbohydrate with little or no interference in the low mass region. The detection limit of bradykinin is about 10 fmol with the signal to noise ratio value of 11.39. The value of the linear regression R² of the calibration curve for the glucose in human urine sample is 0.9992. Furthermore, the practical application of this method is demonstrated by the successful analysis of glucose in urine sample from a diabetic patient.

Acknowledgements
This work was supported by the National Basic Research Program of China(2009CB939701).

Keywords: Analysis, Laser Desorption, Mass Spectrometry, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Mass Spectrometry
Abstract Text
The focus of this research is to explore the effects of surface ligand chemistry on delocalization of excitons and stabilization of surface occupied orbitals of ultra-small semiconductor nanoclusters (SCNCs). To investigate these processes, ligand exchange reactions were performed on octylamine-coated 1.6 nm CdSe nanoclusters with various para substituted phenyldithiocarbamate (PTC) ligands. At their ultra-small size, SCNCs display molecule-like HOMO-LUMO energy gap. We have observed a 580 meV decrease in the core energy, which correspond to a 100 nm red shift of lowest energy absorption peak due to the relaxation of exciton confinement. The mass spectrometry characterization of PTC-coated SCNC showed no change in the core composition. Therefore, the decrease in core energy is due to the exciton delocalization through adjustments to the ligand shell of the NC and not rearrangement of SCNC core. We postulate that the decrease of the SCNC core energy through the introduction of the PTC ligands is a result of mixing of the SCNC valence band with the highest occupied molecular orbital (HOMO) of the X-PTC, resulting in formation of hybrid orbitals. Electrochemical measurements of PTC-coated CdSe nanoclusters were also performed to determine the position of newly formed hybrid orbitals, their energy gap, and columbic interaction energy of electron-hole pair.

Keywords: Electrochemistry, Nanotechnology, Semiconductor, UV-VIS Absorbance/Luminescence
Application Code: Nanotechnology
Methodology Code: UV/VIS
The measurement of pH in biological, chemical, and environmental samples is one of the most widely performed tasks. There is an increasing demand for miniaturized pH sensors for in situ and on-site pH measurements in very small sample volumes. Optical pH sensors have numerous advantages over traditional pH sensitive glass electrodes. However, the sensitivity, sluggish response and short life-time make many optical pH sensors unsuitable for certain analytical applications. To overcome these drawbacks, high concentrations of pH sensitive indicator dyes were encapsulated into ~200 nm diameter nanocapsules with 1 nm thick porous walls. The capsules were immobilized into highly porous polyvinyl alcohol gels for a robust and versatile sensor platform design. The nanocapsule-loaded gels were molded into cylinders or planar thin-films and used as sensing elements in a capillary or a microfluidic flow cell, respectively. The absorbance of the dye molecules inside the nanocapsules as a function of the sample pH was measured with traditional UV-VIS spectrophotometry. These optical pH sensors had high sensitivity, short response times (less than 2 minutes), good reproducibility (< 1% R.S.D) and low signal drift during continuous monitoring experiments. The results demonstrate the feasibility of using the dye-loaded porous nanocapsules to develop optical sensors for real-time pH measurement in very small sample volumes.
### Nanotechnology: Lab-On-A-Chip, Imaging, and Spectroscopy

**Patterned Superhydrophobic/philic Substrates as a Universal Platform for Various Surface-Enhanced Spectroscopic Techniques**

Nanostructures are becoming increasingly popular as a building block for surface-enhanced spectroscopic techniques such as Raman, fluorescence, and localized surface plasmon resonance (LSPR) sensing. One approach based on adsorbing nanospheres on a solid surface, followed by noble metal deposition, has turned out to be effective as well as practical. Appropriate physical parameters vary for a particular spectroscopic technique under question; the sphere diameter may range from 50 to 500 nm while the metal deposition thickness in the range from 5 to well over 100 nm is suitable for Raman and fluorescence. For LSPR sensing, it needs to be optimized in a much narrower range around 15 nm with the sphere diameter around 100 nm. We will give detailed information on appropriate combinations of relevant physical parameters for Raman, fluorescence and LSPR applications based on these surface-adsorbed metal nanoparticles. The same nanoparticle can display other properties useful for a further increase in the signal. We have exploited the lotus effect; when a surface covered by the nanosphere is subjected to a hydrophobic treatment, the surface becomes superhydrophobic. We prepared a substrate covered by silver cap-shaped nanoparticles which are superhydrophobic except for a small central spot for surface-enhancement. When an aqueous sample droplet is placed on the substrate and allowed to dry, molecules suspended in the droplet end up being concentrated on the central spot. The overall effect is signal enhancement through a combination of the optical surface-enhancement and pre-concentration of the molecule.

**Keywords:** Bioanalytical, Nanotechnology, Sample Preparation, Surface Enhanced Raman

**Application Code:** Bioanalytical

**Methodology Code:** Biospectroscopy
Over 16 million units of erythrocytes (ERYs) are stored each year in the US for transfusion. During storage, ERYs undergo many chemical and physical changes, resulting in post-transfusion complications for the patient. Most groups evaluating the properties of ERYs during this storage period use static, non-flow schemes. Here, a fluidic device fabricated by 3-D printing technology is reported that enables ERYs, stored using federal guidelines, to be evaluated in a circulatory flow pattern. There are several key analytical features of this device; 3-D printing results in a device that is rugged and can be re-used for days to weeks. The device enables high flow rates (~50µL/min) without leakage. Furthermore, in addition to six parallel flow channels, there are static calibration wells on the device for simultaneous calibration and/or internal standards. The device is printed as a 96-well plate so that it can be placed directly in a plate reader for high throughput optical measurements. To date, we have determined ATP release from stored ERYs with limits of detection at 52.4 ± 7.4 nM. Our quantitative results from three different channels show no statistically significant difference in terms of sensitivity, detection limit or linearity. We have found that ERYs stored in approved storage solutions released significantly less ATP than those in a modified storage solution being used in our laboratory after 7 days of storage. In fact, on day 1 of storage, the modified solution released 232.3 ± 6.7 nM ATP in comparison to 175.7 ± 15.5 nM ATP for the current, approved solutions (p < 0.03, n = 3 humans).
The ability to perform on-site tests from biological samples has become an important diagnostic tool in many applications. Microfluidic systems are ideal for use in these point-of-care scenarios due to their portability, low cost, and ease of use. Microfluidics also offer the advantage of compatibility with small-volume samples, such as dried blood spots, with minimal sample dilution. This would simplify the sample collection process, eliminating the need for expensive and invasive methods such as venipuncture. Despite these advantages, few methods have been developed to analyze dried blood spots in microfluidic devices. We have designed a microfluidic system compatible with samples dried onto filter paper that uses degassed poly(dimethylsiloxane) pumps to transport fluid through the device. Evacuation of gas from the pump provides the energy required to pull solution through a microfluidic channel when sealed into a device at atmospheric pressure. By evacuating only the pump rather than the entire device, we can perform on-chip extraction of samples from the filter prior to initiating flow for analysis, increasing the amount of material extracted from the paper and improving the detection limits of the device. We are able to generate steady flow rates in these devices for minutes, and can vary the flow rate by altering the dimensions of the pump and the outlet well geometry. This makes the pumping method tunable to the flow required by the desired application.

**Keywords:** Bioanalytical, Flow Injection Analysis, Lab-on-a-Chip/Microfluidics

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Ongoing Enhancements to Chromatographic Methods
Analysis of Multiple Pesticides by Supercritical Fluid Chromatography/Tandem Mass Spectrometry with a Sub-2 Micron Particle Column - A Feasibility Study

Jinchuan Yang  
Waters Corporation

Brian Tyler, Jennifer Burgess, Joe Romano

Abstract Text

GC-MS and LC-MS are common techniques for pesticide residue analysis and both are required due to the wide range of physiochemical properties of pesticides. It has been reported that SFC can be used as a single separation technique for the simultaneous analysis of LC-amenable and GC-amenable pesticides (1). This unique SFC approach provides a simple and convenient solution for multiple pesticide residue analysis. With the new development of UltraPerformance Convergence Chromatography (UPC2), its applicability and potential advantage in multiple pesticide residue analysis has been investigated. UPC2 is a separation technique that uses compressed carbon dioxide as the primary mobile phase. It takes advantage of the unique physical properties of compressed carbon dioxide (at or near supercritical state), sub-two micron particle chromatography columns and an advanced chromatography system design to achieve unique selectivity, high efficiencies and speed. In this work, we present a feasibility study using UPC2 with ultra sensitive tandem quadrupole MS/MS detection for 18 pesticides with a wide range of polarities (logPow=-4.6 to 7.1) and molecular weights (112-889). This study focused on UPC2 separation, interfacing with MS, and MS/MS detection. The sensitivity and repeatability of the multi-pesticide residue analysis in the presence of common food matrices, such as spinach, wheat flour, and apple juice will be presented.

Keywords: Mass Spectrometry, Pesticides, SFC
Application Code: General Interest
Methodology Code: Other (Specify)
For many years, the presence of a second retention mechanism has been considered a curse for HPLC columns. With reversed phase columns, numerous solutions, both simple and complex, have been developed to minimize anything other than hydrophobic interactions. However, several new stationary phases are now available that, by design, exhibit multiple retention mechanisms. Many of these phases are referred to as "HILIC" but the presence of multiple retention modes is the key consideration for this presentation. The popularity of these phases has been hindered by a poor understanding of these mechanisms, and the columns are often considered to be unpredictable. A simple screening approach to identifying the two most likely separation mechanisms will be shown and demonstrated for columns of different chemistry and from different manufacturers. We will then show how the presence of a second separation mechanism can be used to advantage in developing separations of compound classes from each other, using a basic understanding of the behavior of each retention mechanism.
Ongoing Enhancements to Chromatographic Methods

A Refractive Index Detector for UPLC

UPLC is a separation technique that employs analytical LC columns packed with sub-2 μm particles and an LC system which has been holistically designed to maximize the separating power of those columns. The UPLC technique has become increasingly popular due to the greatly enhanced separations power and shorter analysis times it can deliver. This has led to an interest in extending UPLC to those applications which employ alternative detection to the commonly used UV/PDA, MS and ELSD. Refractive Index (RI) is a robust device that approaches the ideal of universal detection. A chromophore is not required; detection of compounds eluted from a column is based upon the difference in refractive index between the analyte and the background mobile phase. In many laboratories, RI is the detector of choice when analyzing molecules lacking a chromophore because it is simple to use and needs no equilibration between injections. There is no need for a clean nitrogen supply as is required for ELSD, which provides an economic benefit and is ideal for a manufacturing facility. RI detection provides excellent repeatability and shows a linear response for quantification. RI detectors measure the differential refraction between a sample flow cell and a static reference cell filled with mobile phase and have the particular challenge in that anything that can create changes in mobile phase density, and thereby the refractive index of the mobile phase as it passes through the sample cell, must be carefully controlled. A Refractive Index Detector suitable for UPLC separations must maintain the fidelity of the chromatographic bands generated by the UPLC; this requires a significant reduction (X10) in both tubing and cell volume while maintaining thermal stability. HPLC/RI applications from food sugars, saccharides, pharmaceutical QC, and polymer analysis are transferred to UPLC/RI with significant gains in assay through put and resolution.

Keywords: HPLC Detection, Liquid Chromatography
Application Code: Quality/QA/QC
Methodology Code: Liquid Chromatography
Ongoing Enhancements to Chromatographic Methods

Comparison of UHPLC and Superficially Porous Particles in HPLC

LC method development faces the challenge of simultaneous maximization of resolution and detection limits in the shortest time within given pressure constraints. One of the key factors enabling high resolution and speed in LC is a reduction in particle diameter. Two recent innovations, the use of very small totally porous particles at extremely high pressures (UHPLC) or the use of superficially porous particles (ultra-small particles bonded onto the surface of large, non-porous particles), have opened up new possibilities for achieving high resolution and high speed analyses.

In this paper, we contrast the performance of conventional columns (3.5 μm particles) with those from these newer technologies -- UHPLC (1.7 μm) and superficially porous (2.7 μm) -- under a variety of isocratic and gradient chromatographic conditions. Practical issues related to achievable improvements in chromatographic performance (e.g. analysis time, efficiency, loadability, and resolution) will be considered. Each column type has its strengths and weaknesses. We will discuss approaches for choosing the right particle type and column configuration for a given application.

Experiments were funded by Axion Analytical Labs, Inc.

Keywords: High Throughput Chemical Analysis, HPLC, HPLC Columns, Liquid Chromatography
Application Code: High-Throughput Chemical Analysis
Methodology Code: Liquid Chromatography
Ongoing Enhancements to Chromatographic Methods

Novel Hybrid Metal Organic Framework-Organic Polymer Monolith for Chromatographic Application

Room temperature ionic liquids (RTILs) are considered promising green solvents because of their high thermal, chemical stability and negligible vapor pressure. Similarly, using microwave heating for polymeric synthesis has the advantages of reduced reaction time and increase yield and reproducibility. In this study, a novel hybrid metal organic framework (MOF)-organic polymer monoliths prepared via a 5-min microwave-assisted polymerization of methacrylate based monomers with the addition of various weight percentages of porous MOF were developed as stationary phases for capillary electrophromatography (CEC) and nano-liquid chromatography (nano-LC). The inclusion of MIL-101(Cr) MOF provided very high surface areas, meso- and nano-sized pores and aromatic terephthalate moieties in the produced MOF-polymer monolith. This hybrid MOF-polymer column exhibited high permeability, with almost 800-fold increase compared to MOF packed column, and efficient separation of various analytes (xylene, chlorotoluene, cymene, PAHs, aromatic acids and BSA trypsin digested peptides) either in CEC or nano-LC. This work demonstrated high potentials for MOF-organic polymer monolith as chromatographic stationary phase.

Keywords: Capillary Electrophoresis, Capillary LC, HPLC Columns
Application Code: General Interest
Methodology Code: Liquid Chromatography
Lubricants, such as motor oil, are used to reduce friction in vehicles, tools, and other moving parts. These lubricants usually consist of a base oil, and additives to enhance the properties of the oil. Types of additives are reliant upon the properties needed for the specific lubricant. For instance, additives may provide oxidation resistance, improve viscosity, resist corrosion, or reduce wear. Antioxidants can be phenols and amines; halocarbons and thiophosphates have good antiwear properties; and detergents, like calcium and magnesium sulfonates are used to remove oil impurities. Thermal treatment of lubricants as a sample preparation technique creates the extraordinary ability to release additives without using solvent extraction. Lubricants can be heated to a low temperature to volatilize smaller additive molecules directly to the gas chromatograph. Afterwards, the remainder of the lubricant can be heated at a higher, pyrolysis temperature, to break larger molecules into smaller, volatile fragments. Using pyrolysis-GC/MS equipment, we will thermally treat a variety of lubricants to investigate additives. We will study lubricants such as motor oil, transmission fluid, cutting fluid for machinery, and household lubricants, like WD-40®.
An on-going demand within the field of gas chromatography is the development of new classes of stationary phases that are capable of producing low column bleed at elevated temperatures. Ionic liquids are an interesting class of ionic solvents which exhibit a number of physico-chemical properties including negligible vapor pressure, high thermal stability and broad liquid ranges. These factors have allowed ILs to be effectively used as stationary phases in gas-liquid chromatography. Identifying ILs that exhibit very high thermal stability as well as minimal degradation products at elevated temperature would expand their application into methods employing GC/MS. Recently, tetraaryl-phosphonium (TAP) based ILs have been found to be a new class of ILs that exhibit superior thermal stability compared to many other classes of ILs. The implementation of this class of stationary phases in gas chromatography will be discussed. Factors such as etching of the capillary wall and other surface modifications that give rise of high column efficiency will be presented. In addition, the long term effect of column temperature on the stability of the stationary phase as well as an evaluation of the solvation properties of the stationary phases will be discussed.

Abstract Text

Keywords: Gas Chromatography, Gas Chromatography/Mass Spectrometry, GC Columns, Separation Sciences

Application Code: General Interest

Methodology Code: Gas Chromatography
The chromatogram is like a fingerprint. If you can read the chromatogram by looking at peak shapes, retention, base line and by comparing with “normal” situation, you have a good chance to solve problems and improve the analysis. A ghost peak is a peak that is showing up, but is not supposed to be there. Sometimes it is referred as a “system” peak. Ghost peaks can be created in many ways. It’s a component that is added/created somewhere in the system, it is injected/trapped/focused onto the column, and will elute. Problems will escalate if a ghost peak interferes with an analyte that has to be quantified. Sources for ghost peaks can be sample vials, gloves, syringes, reagents, carrier gas, tubing, the injection port, operation, memory effects and even the column-phase itself. In this poster the most prominent contributions to ghost peaks will be discussed by showing practical examples and how to check for.

Keywords: Gas Chromatography, Gas Chromatography/Mass Spectrometry, Teaching/Education
Application Code: General Interest
Methodology Code: Gas Chromatography
Optimizing Resolution in Reversed-Phase UPLC Methods Development with Automatic pH Selection

Retention in reversed-phase chromatography can show significant changes with slight variations in pH. The adjustment of pH can also or similarly serve as a very important and useful screening tool to adjust retention and selectivity. Choosing the optimum pH is difficult for a multi-component sample representing a wide range of properties because we cannot predict optimum separation pH. We have developed an efficient automation of this screening process by integrating the blending of stock buffers by the liquid chromatography system to deliver mobile phases of different pH and ionic strength. To extend the utility of automated blending of stock buffers by the system to compounds with different functional groups often requires selection of a different buffer system to cover a different pK range. We have developed a protocol that can be applied to unknown compounds exploiting combinations of buffers. Such a multi-buffer system can be used in different applications such as impurity profiling, stability testing and can be used with unknown compounds that have a mix of analytes sensitive to different ranges in the pH spectrum.

We have developed and validated two buffer systems: one consisting of non-volatile buffer components for methods development of reversed-phase LC separations and one consisting of volatile buffer components for LC-MS methods development. These two buffer systems provide buffering capacity over a wide pH range. Using these two validated buffer systems, we demonstrate the effect of pH change on chromatographic properties of acidic, basic and neutral compound mixtures. This systematic and automated screening protocol using a single defined buffer system will prove to be useful for reversed-phase method development.

Keywords: Chromatography, HPLC, Liquid Chromatography, Optimization
Application Code: General Interest
Methodology Code: Liquid Chromatography
Hydrophilic interaction chromatography (HILIC) is gaining popularity in liquid chromatography, particularly for its ability to retain and separate small polar analytes—an area where common reversed-phase liquid chromatography (RPLC) methodology often fails. This novel mode of chromatography results in unique selectivity compared to traditional RPLC. Water is used as the strong eluting solvent with a polar stationary phase, causing compounds to elute from least polar to most polar; this often reverses elution order as compared to RPLC. This presentation will address the use and best practices of HILIC stationary phases with regards to optimal polar compound retention. Stationary phases, solvent strength and buffers types will be addressed with respect to their unique impact on hydrophilic interaction chromatography, as well as advantages and disadvantages to the technique.
Environmental education curricula were planned by using acid rain research results for elementary school level through university level. For elementary schools, rain samples collectors could be placed, and samples could be collected and analyzed for pH as well as conductivity by students themselves under the help from instructors. Snow samples were collected and analyzed by grade 6 students themselves in an elementary school. It would be great opportunity for them to see if rain or snow could be affected by environmental problem. For university students, there are many things to be learned from the results. They could analyze samples and discuss the results by themselves. With the aid of the spreadsheet, computer program, they could review all the results from 2006 through 2013, for eight years, based on thousands of data. From elementary school through university, students would understand the effect of environmental problem to the acid rain by themselves with these research and education program. Rain samples have been collected and analyzed since 2006 through 2013 in the urban area of Sapporo, in the northern part of Japan. All samples have been measured for pH and conductivity, and some of them have been analyzed by ion chromatography and atomic absorption spectrometry for anions and cations. Most of rain samples collected were acid rain; their pH were below 5.6, but some of them showed pH above 5.6; the “basic” rain. According to the wind direction and seasonal point of view, those "basic" rain could be derived from chemical fertilizers added to the farmland.

Keywords: Education, Environmental
Application Code: Environmental
Methodology Code: Education/Teaching
Dissolution testing is a quantitative analytical technique used to measure the release rate of ingredients from tablets and capsules. This lab experiment teaches students how to compress tablets from a mixture of powders and challenges students to build their own apparatus to measure the dissolution rate of their tablets. Each lab group was able to complete the experiment successfully and elucidate two predicted trends in dissolution rate from a set of five tablets. Students learned the fundamentals of UV spectroscopy, data handling and calibration, and sampling while gaining insight and experience regarding an important test used in the pharmaceutical and dietary supplement industries.
Analytical chemistry is increasingly becoming an integral part of all science and engineering disciplines, and it plays a vital role in broader societal concerns. Thus, today’s analytical chemistry education must be more relevant to modern analytical laboratory practices. Although traditional undergraduate chemistry curricula provide a solid foundation in the fundamental principles of analytical chemistry, they do not formally value practical skills that enable students to adapt and be successful in today’s rapidly changing and competitive analytical workplace. To bridge the current gap between “real work” experiences and university training in analytical chemistry, the author has integrated real world chemical separation research projects into analytical chemistry curriculum to prepare our students academically for what the real world wants from them. The integrated research projects challenged students to think creatively and improved their skills in communication, teamwork, and problem-solving. The details of this innovative curriculum project will be given in the presentation.

Keywords: Bioanalytical, GC-MS, HPLC, Separation Sciences
Application Code: Bioanalytical
Methodology Code: Education/Teaching
Technology can have a positive influence on learning, and this is changing how we design and deliver our courses. This paper is based on an ongoing research program examining the implementation of smartphones Apps to improve student learning outcomes. Our objective is to explore how to take advantage of the Apps to help students to learn chemistry.

It is imperative for learning chemistry to understand how the periodic table of elements is organized and how to read information from it. There are more than 60 Apps for periodic tables. Other Apps cover all the concepts of chemistry from the basic of naming compounds and writing formulas to more advanced topics like the interpretation of spectroscopy data. Many Apps provide lessons on general chemistry and organic chemistry with exercises and quizzes to help students master the concepts and evaluate their level of comprehension. There are Apps that can view, edit and build molecules in 2D and 3D, others with screencasts for analytical chemistry calculations of molarity and dilutions, games for experiments, dictionaries of chemistry terms, and titration simulators. Others provide information in the form of flashcards. Students are asked to revise the Apps and to practice with them for homework. We try to make homework more interesting and relevant to students. By practicing with the Apps students get a better understanding of chemistry and feel more confident. Questionnaires were answered by the students about the use of smartphones for learning. Most students made positive comments about what they thought of the use of Apps for learning chemistry. More than 80% of students consider the use of Apps to be fun and useful.

Keywords: Computers, Informatics, Teaching/Education
Application Code: General Interest
Methodology Code: Education/Teaching
The inverted classroom model supports an active-learning experience in the classroom. Rather than spend class-time lecturing to students, a lecture is prerecorded and made available to the students for viewing prior to their arrival in class. Class-time is then spent applying the concepts introduced in the video, where students work in groups, asking questions when needed and enjoying a more-personalized learning experience. This learning-by-doing approach replaces the passive lecture-based learning with an active, student-centered experience where students can direct the learning process based on their needs. The course content typically covered in the general chemistry curriculum requires a strong emphasis on problem solving skills that can be effectively targeted with this approach. Thus the introductory chemistry course serves as a natural fit for this type of learning. From the perspective of a first-time “flipper”, this presentation will focus on a number of lessons-learned, as well the use of various technological tools that were utilized to overcome a variety of challenges that became apparent during the course. Additionally, student performance was tracked and assessed under the inverted model to enable a direct comparison to a traditional, lecture-centered classroom taught by the same instructor.
Microcontrollers are powerful programmable devices that can automate equipment and perform data acquisition. Over the past decade increased memory storage has enabled the use of object-oriented programming languages on these devices, thereby making them easier to use. More recently commercial microcontroller boards have become available loaded with connectors and accessory components for enhancing communication between the microcontroller and other equipment. Because these boards are well-supported on-line and are inexpensive (typically $30-$50) they offer a convenient and versatile approach to interfacing computers to lab experiments. This presentation will demonstrate several example applications for teaching lab automation and basic electronics in the context of an instrumental analysis course. Interfacing projects include acquisition and manipulation of photometric data, an auto-titration system, a PCR thermal cycler, control of stepper motors for syringe pumps and sample collection. Classroom exercises and device limitations will also be discussed.

**Keywords:** Automation, Computers, Education, Instrumentation

**Application Code:** General Interest

**Methodology Code:** Education/Teaching
Passive air sampler for the determination of atmospheric concentration of NO$_2$ has been often applied to environmental education for recognizing a present status of local air quality. However, students and/or children tends to regard NO$_2$ emission sources such as factory chimney, automobile exhaust and tobacco smoke, as social evils excessively, when poor discussions were made on the monitoring data. The purpose of this study is development of new educational opportunities aiming to cultivate sense of environmental forensics including the ability of discussion on the emission and behavior of air pollutants for further promotion of better pollution control. Presenters then developed a class on the practice of monitoring of NO$_2$ together with its precursor NO in air using a passive air sampler, and have offered to high school students as a special class and both graduate and undergraduate university students as a session of the regular class. In the class, students assemble passive air samplers for NO and NO$_2$ by themselves, expose them in indoor and outdoor air of their own houses, determine the concentrations of both gases by absorption spectrophotometry, and subsequently discuss the levels, indoor/outdoor ratio and NO/NO$_2$ ratio for describing the pollution mechanism in their houses. By reviewing reports from students, most of them successfully discussed emission sources and behavior of NOx by relating the data with a housing structure and location. Furthermore, some university students incorporated this methodology into their thesis. These results met the learning objective of this class.

Keywords: Environmental Analysis, Environmental/Air, Forensics
Application Code: Environmental
Methodology Code: Education/Teaching
This presentation describes the design and implementation of a blended analytical chemistry course. The objective of the approach was to improve students’ quantitative problem solving and conceptual understanding of the material in a junior-level undergraduate analytical chemistry course. The redesigned course was inspired by the flipped classroom concept. Lecture materials were made available online to accompany a free online textbook. The online materials incorporated text, graphics, and video clips embedded in publicly available Prezi presentations. Prezi presentations provide a mechanism for guiding learners through the self-study learning path paved with animated concepts linked in a non-linear knowledge structure while allowing them to “dip” in and out of materials as they wish. The classroom portion of the course met for two, 1 hour 15 minute periods per week. The class period include short tests to ensure students were familiar with assigned content, a short review and question session, and one or two multiple choice questions to test knowledge of concepts. The majority of classroom time involved students working in small teams to solve online homework questions with the guidance of the instructor. The instructor interacted with students outside of class time via a dedicated Facebook group. The advantage of this approach is that it allows the instructor to focus less time on content and more on problem solving which students tend to find more challenging. It also increases direct student/instructor interaction. Students were assessed using graded online homework, short-answer written tests and a standardized American Chemical Society (ACS) multiple-choice test. The success of the approach was assessed by pre- and post-course questionnaires and student performance on the ACS test compared to previous years. This presentation will report on the findings of this approach in terms of student learning and attitude.

Keywords: Education
Application Code: General Interest
Methodology Code: Education/Teaching
With the recast of the European Union’s RoHS Directive 2002/95/EU restricting Lead, Cadmium, Hexavalent Chromium, Mercury and Polybrominated Biphenyls (PBB) and Polybrominated Diphenylethers (PBDE) in electrical and electronic equipment (EEE), manufacturers must ensure their products meet the new requirements to be sold in the marketplace. As of July 22, 2014 and July 22, 2016 when manufacturers place a CE Mark on medical devices and in vitro diagnostics, respectively, they are confirming that all appropriate measures have been taken to ensure each product meets the new Directive as well as the requirements of all relevant CE Directives.

Published in the Official Journal in July 2011 and transposed into EU national law in January 2013, the recast RoHS Directive 2011/65/EU, also known as RoHS 2, contains many changes in terms of scope, definitions and newly introduced methodologies and procedures.

It is the sole responsibility of the manufacturer to compile the "technical file" that enables RoHS 2 assessment of product conformity. This means considerably more work for manufacturers, importers and distributors regarding the documentation and monitoring of medical devices, requiring a continuous exchange of information in the entire supply chain. Documents in a RoHS 2 technical file include conformity risk assessments, declarations of compliance, material declarations, audit results, chemical analysis results and explanations of why test reports are not included when absent.

During this presentation, Joe Langton, business service line leader for the chemical industry at Intertek, will provide attendees with a greater understanding of RoHS 2 requirements. He will also discuss why it is important for manufacturers, importers and distributors to fulfill the obligations of the recast RoHS Directive.
This talk will describe some work from Northwestern on constructing, tailoring, and utilizing nanostructured electrodes in order to facilitate photoelectrochemical energy conversion -- both sunlight-to-electrical energy and, if time permits, sunlight-to-chemical energy. Much of the work to be presented will focus on how atomic-layer deposition can be used to build electrode structures that are effective for energy conversion as well as provide experimental insight into what controls the kinetics and dynamics of productive versus unproductive reactions at photo-electrode/solution interfaces.
The Hamann group is interested in the possibility of utilizing very inexpensive materials to achieve efficient solar energy conversion and storage. The dye-sensitized solar cell (DSSC) is a particularly interesting system as it proffers the possibility of efficient solar energy conversion using electrodes comprised of TiO2 nanoparticles. The 600 meV driving force required for efficient dye regeneration with the I3-/I– redox shuttle is the primary loss mechanism which has limited the overall energy conversion efficiency of conventional DSSCs. We will present recent results of investigating the possibility of replacing the nearly ubiquitous I3-/I– redox shuttle with one-electron outersphere cobalt-based redox shuttles. One particularly promising example that will be discussed is the low spin cobalt(II) complex, cobalt bis–trithiacyclononane, [Co(ttcn)2]3+/2+. This unique cobalt complex redox shuttle is stable, transparent, easy to synthesize from commercial ligands, and has attractive energetic and kinetic features for use in DSSCs. A series of photoelectrochemical and spectroscopic measurements were employed to determine to what extent regeneration and recombination are limiting processes. In addition, these combined results demonstrate the possibility of advancing this exciting technology as well as highlighting some of the roadblocks.

**Keywords:** Energy, Fuels\Energy\Petrochemical

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

**Methodology Code:** Electrochemistry
Joe Hupp has been doing wonderful science for a few decades, and this talk will be based on some modeling in support of what he has done. Going back to electron transfer and proceeding through MOF’s and voltaics, we will examine some theoretical constructs and simulations, to help understand what Joe has taught us.

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

**Application Code:** Materials Science

**Methodology Code:** Computers, Modeling and Simulation
This presentation covers our recent work with liquid metal electrodes as a versatile medium for (nano)material syntheses. An initial discussion will be given covering the historical uses of liquid metal electrodes in electroanalytical chemistry, highlighting long recognized limitations in the analysis of stripping voltammetry of dissolved metals. This same 'problem' is a phenomenon we are now exploiting as a controllable covalent semiconductor crystal growth method. Data will be presented that shows the direct electrodeposition of desirable Group IV and III-V semiconductors with liquid metal electrodes. Through a process we refer to as an electrochemical liquid-liquid-solid (ec-LLS) crystal growth, we demonstrate the capacity to electrodeposit both crystalline semiconductors AND semiconductor devices. A final discussion will be given on the utility of this process for developing advanced nanowire-based analytical platforms at low cost with benchtop equipment.

Abstract Text

Keywords: Electrochemistry, Electrodes, Energy, Nanotechnology
Application Code: Materials Science
Methodology Code: Electrochemistry
The development of advanced materials and technologies to efficiently convert and store energy directly into electricity is of urgent importance due to increasing energy demands of an ever-growing world population and decreasing energy reserves. However, tremendous scientific challenges remain before successful implementation of any number of competing energy technologies such as solar cells, fuel cells, and batteries. The materials, interfaces, and device architectures currently being explored are very challenging to interrogate by ensemble-averaging, bulk experimental methods since they do not exhibit long-range order or homogeneity, contain unique nano-morphological features and possess non-uniform chemical compositions and defect chemistry. This presentation will summarize a few materials design strategies for optimizing the performance of redox-active materials for use in electrochemical energy storage applications and highlight the development of high resolution analytical tools for studying charge transfer processes at electrode interfaces. Information obtained from these studies provides fundamental understanding of electron and ion transfer processes and degradation mechanisms for materials utilized for electrochemical energy storage.
Cocamidopropylbetaine is a common surfactant used in personal-care products. Dimethylaminopropylamine and lauramidopropyldimethylamine are two chemicals applied in the synthesis of cocamidopropylbetaine and known to be present in personal-care products containing cocamidopropylbetaine. Even though cocamidopropylbetaine is safe in the products as long as it is formulated to be non-sensitizing based on a quantitative risk assessment, dimethylaminopropylamine and lauramidopropyldimethylamine are recognized as having the potential to cause adverse reactions in human skin. A rapid and sensitive core-shell HILIC-UPLC-MS/MS method has been developed to chromatographically determine dimethylaminopropylamine and lauramidopropyldimethylamine. Corresponding isotopically labeled analogues were selected as internal standards to correct for recovery and matrix effects. The preliminary data show that dimethylaminopropylamine has a linear range from 2 to 500 ng/mL and lauramidopropyldimethylamine has a linear range from 0.2 to 50 ng/mL. Both have correlation coefficients (r) greater than 0.995. Ongoing work will include validation of the UPLC-MS/MS method for cosmetic products and performance of a limited survey on at least 30 commercial personal-care products to determine the residual levels of these two chemical intermediates.

Keywords: High Throughput Chemical Analysis, HPLC, Liquid Chromatography/Mass Spectroscopy, Validation

Application Code: High-Throughput Chemical Analysis

Methodology Code: Liquid Chromatography/Mass Spectrometry
For decades, analytical chemists working on the topics of food and environmental contaminants have postulated that advanced mass spectrometry (MS) tools would meet detection needs for hundreds of analytes in complex matrices in a fast, simple, and rugged analysis without using chromatography. Some successes have been achieved depending on the specific applications, but analytical separations provide much needed selectivity in common food applications, even when using modern instruments capable of MS/MS and high-resolution MS. Isobaric and isotopic interferences of molecules with the same ion transitions and/or molecular formulae are usually separated by the orthogonally-selected means of gas (GC) and/or liquid chromatography (LC). In recent years, Nanita et al. in a series of publications have demonstrated feasibility of flow injection (FI)-MS/MS for dozens of pesticides in different complicated matrices, including foods, soil, and blood. However, for regulatory and other food monitoring purposes, hundreds of chemical residues typically need to be screened, quantified, and identified at <10 ng/g in hundreds of different foods. Initial studies on sample preparation based on the “quick, easy, cheap, effective, rugged, and safe” (QuEChERS) approach have been completed to provide relatively clean extracts, and a triple quadrupole Q-Trap instrument is being evaluated for use in FI-MS/MS. The exceptionally low instrumental detection limits allow >100-fold dilution of extracts to still meet <10 ng/g analyte detection needs. Optional use of in-source ion mobility spectrometry also provides greater selectivity to avoid matrix interferences without chromatography.
**On-Site Screening for Plasticizers, Maleic Acid, Melamine, and Residual Pesticides in Tainted Foods via Mobile Ambient Mass Spectrometry (MAMS)**

On-site screening for chemical compounds in tainted foods is an efficient strategy in order to reduce food safety risks to the general public. For this purpose, we have developed a high-performance movable mass spectrometer by installing a commercial mass spectrometer with a thermal desorption/electrospray ionization unit (TD-ESI) on a mobile vehicle. This mobile ambient mass spectrometry (MAMS) system comprised of a commercial mass spectrometer equipped with a TD-ESI source, gas generator, and power supply. The AMS was set on a moving cart, and triple-shock absorbers were added to protect the system from damage during transportation. Liquid or solid aliquots were obtained from samples using a metal probe, which was inserted in a preheated oven to thermally desorb sample analytes. The desorbed analytes were conducted into an electrospray/plasma plume for post-ionization. Both polar and nonpolar chemical compounds can be ionized and subsequently detected by an ion trap mass spectrometer.

The mobile ambient mass spectrometry (MAMS) system can be used to screen for plasticizers in tainted wines and beverages. The time required to complete an analysis (including sampling, ionization and detection) is less than 5 seconds. The limit of detection (LOD) of plasticizers such as di-ethyl hexyl phthalate (DEHP) in sport drinks and wines and diisononyl phthalate (DINP) in beverages was estimated to be below 500 ppb. Residual pesticides on fruits and vegetables were screened using this MAMS system, where carbendazim, thiabendazole, and imazalil were the three most common residual fungicides detected on imported and local apples and oranges. The LOD of thiabendazole and imazalil on the surfaces of fruits was estimated to be below 100 ppb. The technique was also used to detect trace amounts of maleic acid in traditional Taiwanese foods. The LOD of maleic acid in starch solution was found to be below 5 ppm.

**Keywords:** Food Science, Mass Spectrometry, Pesticides, Sampling

**Application Code:** Food Science

**Methodology Code:** Mass Spectrometry
In the recent past, allergen analysis was traditionally performed using antibody based technologies (ELISA/LFD) or, to a lesser extend, DNA-based technologies (PCR, real-time PCR). However, it is well known and documented, that these technologies have a number of drawbacks which can lead to false positive and false negative results. While false positive results have a financial impact on the producer and brand name, false negative results can have severe consequences for the allergenic patient. With the onset of mass spectrometry, and the comparison with existing technologies like ELISA and PCR, some of the shortcoming of traditional technologies (PCR, ELISA) became even more obvious. This presentation will put mass spectrometry analysis into perspective to ensure food safety and present a case where the erroneous results produced by immunological techniques had been uncovered using mass spectrometry.
This paper presents an application of ultra-high performance liquid chromatography (UHPLC) electrospray ionization (ESI) quadrupole Orbitrap high resolution mass spectrometry (Q-Orbitrap) for determination of ~ 450 pesticide residues in fruits and vegetables, and 100 antibiotics residues in milk. Pesticides or antibiotics were extracted from samples using the QuEChERS (quick, easy, cheap, effective, rugged and safe) procedure. UHPLC/ESI Q-Orbitrap MS (i.e. full MS scan) acquired full MS data for quantification, and UHPLC/ESI Q-Orbitrap dd-MS2 (i.e. Data Dependent scan) obtained product-ion spectra for confirmation. UHPLC/ESI Q-Orbitrap MS quantification was achieved using matrix-matched standard calibration curves. The method performance characteristics that included overall recovery, intermediate precision and measurement uncertainty were evaluated according to a nested experimental design. Overall, the UHPLC/ESI Q-Orbitrap has demonstrated its great performance for quantification and confirmation of pesticide residues in foods, and antibiotics in milk. Q-Orbitrap can provide low ppb sensitivity and is suitable for most regulatory requirements in the analysis of pesticide and antibiotic residues in food.
Boron-doped diamond (BDD) electrodes are attractive material, because of their wide potential window, low background current, chemical inertness, etc[1]. In these years, we have reported several examples for electrochemical sensor applications[2]. Here, we report some recent examples of electrochemical sensor application of BDD such as ozone [3], pH [4], in vivo detection of neurotransmitter in monkey brain [5], and in vivo detection of glutathione for assessment of cancerous tumors [6] using BDD microelectrodes. Furthermore, other applications such as organic synthesis [7], ozone generation are also shown.

In 2007, we have reported in vivo detection of dopamine in a mouse brain by using BDD microelectrodes with the tip diameter of about 5µm [2g]. Here, the BDD microelectrodes were successfully prepared, and reward-induced burst firing of dopaminergic neurons has mainly been studied in the primate midbrain [5]. In primate brains during Pavlovian cue-reward trials, a sharp response to a reward cue was detected in the caudate of Japanese monkeys.

Recently, the in vivo electrochemical detection of the reduced form of glutathione (GSH) using BDD microelectrode for potential application in the assessment of cancerous tumors is presented. In vivo GSH detection measurements have been performed in human cancer cells inoculated in immunodeficient mice. These measurements have shown that the difference of GSH level between cancerous and normal tissues can be detected.

References
I will describe progress in nanoscale magnetic imaging using atom-like Nitrogen-Vacancy (NV) color centers in diamond. NV-diamond provides an unprecedented combination of magnetic field sensitivity and spatial resolution in a room-temperature solid due to the remarkable properties of NV centers, including long electronic spin coherence times, optical spin polarization and read-out, a large Zeeman shift of the spin transitions, and the robust physical properties of diamond in a wide variety of forms (bulk crystals, films, nanocrystals, etc.). Promising applications include sensing and quantum control of individual electron and nuclear spins, and nanoscale imaging of magnetic fields from biological cells under ambient conditions.

Keywords: Biosensors, Imaging, Magnetic Resonance, Nanotechnology
Application Code: Other (Specify)
Methodology Code: Other (Specify)
With the expanding use of nanomaterials in consumer products, there is increased interest in understanding the environmental fate and toxicity of nanoparticles. One challenge is that most nanoparticles are chemically degraded in environmental and biological environments, making it difficult to know the chemical form of the nanoparticle. Diamond nanoparticles can be covalently functionalized with a range of different molecular ligands, providing the ability to tune its properties in a wide-ranging manner. We have been investigating the chemical modification of nanodiamond and its use as a potential non-toxic tracking agent in biological and environmental studies. Nanodiamond can be detected using a variety of methods, including confocal Raman microscopy and via fluorescence for the N[sub]V[/sub] center, a naturally occurring fluorescent defect. The challenges, opportunities, and progress in use of nanodiamond for environmental studies, including in situ characterization within single- and multicellular organisms will be discussed.
Advances in Diamond Based Sensing and Analysis

Diamond Microelectrodes for Neurochemical Studies in Human Tissues

Conducting diamond is proving to be a useful new microelectrode for in vitro electroanalytical measurements of neurotransmission and for electrochemical detection coupled with capillary electrophoresis. Interest in this electrode material has arisen because of its outstanding properties: (i) hardness, (ii) low, stable and pH-independent background current, (iii) morphological and microstructural stability over a wide range of potentials, (iv) good electrochemical responsiveness for multiple redox analytes without any conventional pretreatment and (v) weak molecular adsorption of polar molecules that leads to a high level of resistance to response deactivation and electrode fouling. Diamond electrodes have advanced in recent years from being simply a scientific curiosity into an excellent material for electroanalysis. The microelectrodes are prepared by depositing a thin film of electrically conducting, polycrystalline diamond on a sharpened 40 or 76-µm diam. platinum wires. Polypropylene is used for insulation in a manner that produces a conically-shaped microelectrode. In this presentation, we will describe the preparation of these microelectrodes, discuss their basic electrochemical properties and highlight how they can be used to study neurochemical signaling processes in human tissues. Specifically, we will highlight our efforts to understand how serotonergic signaling in the small intestine mucosa is affected in obesity. This work utilizes tissues obtained from patients undergoing gastric by-pass surgery along with tissues from an animal model of obesity.

Keywords: Bioanalytical, Electrochemistry, Electrode Surfaces, Electrodes
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Advances in Diamond Based Sensing and Analysis

Electrochemical X-Ray Fluorescence (EC-XRF): A New Technique for Heavy Metal Detection at Sub-ppb Levels

Anodic stripping voltammetry is a well-known electrochemical technique for the detection of heavy metals in solution at ppb and lower levels. It relies on an electrochemical preconcentration step followed by stripping of the metal from the surface. The peak intensity (or area) of the stripping peak and its position on the voltage axis are used to quantify the concentration and identify the metal, respectively. Traditionally Hg was used as the support electrode, forming amalgams with the different heavy metals. However, as Hg has now been banned solid electrodes must be used instead, which can complicate interpretation of the analytical signal as it has been shown the morphology of the deposit on the solid surface will affect the stripping peak position and shape [1,2]; this becomes further problematic in mixed metal solutions. In this talk we showcase the use of electrochemical X-ray fluorescence spectroscopy (EC-XRF) for the detection of heavy metals in solution. The ideal support electrode for this work is electrochemically optimised conducting boron doped diamond (BDD)[3]. EC is employed to preconcentrate the metal of interest on the BDD surface, whilst the XRF component is used to uniquely identify the metal and quantify the solution concentration (Figure 1). We illustrate the capabilities of EC-XRF through measurements of different heavy metals in both single and mixed metal solutions at the sub ppb level.

References

Keywords: Electrochemistry, Electrode Surfaces, Environmental/Water, X-ray Fluorescence
Application Code: Environmental
Methodology Code: Electrochemistry
Advances in Raman Spectroscopy

Using Deep-UV Resonance Raman Spectroscopy to Monitor Protein-Lipid Interactions

Structural analysis of membrane proteins lags well behind their soluble counterparts, largely due to the unique challenges presented by the lipid environment. However, structural characterization of membrane proteins is becoming an imperative as they make-up over fifty percent of the drug targets. Deep-UV resonance Raman (UVRR) spectroscopy has been used widely to characterize the structure and dynamics of soluble proteins. Recently, deep-UVRR has been extended to lipid-solubilized proteins with little interference from the lipid environment. It was found that the amide III, S and II modes are sensitive to the secondary structure of the protein, while the amide I mode is highly sensitive to protein environment. A series of proteins varying in size, secondary-structure and number of transmembrane helices have been characterized using deep-UVRR. The results of these studies will be presented.

Keywords: Raman
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Advances in Raman Spectroscopy

Low-Wavenumber Stokes and Anti-Stokes Raman Microscopy for Pharmaceutical Tablet Characterization

Discrimination between polymorphs of an active pharmaceutical ingredient (API) in a pharmaceutical product is important because an undesired API crystal form may have different bioavailability or stability characteristics than the desired form. Micro-Raman mapping has proven useful for nondestructive, spatially resolved identification of API polymorphs even at low API levels in the drug product.

Raman spectral polymorph discrimination is usually based on small band shifts in the fingerprint region (400-1800 cm$^{-1}$). These shifts result from functional groups, such as a carbonyl group, experiencing different microenvironments in the different crystal forms. Low-wavenumber Raman bands (-200 to 200 cm$^{-1}$) result from larger scale motions, such as deformation of the molecular skeleton or even the whole unit cell. Since low-wavenumber Raman bands are more directly related to the entire crystal structure than vibrational bands from small functional groups, they often improve API polymorph discrimination. The anti-Stokes segment of this spectral region provides additional Raman intensity, though no additional spectroscopic information, that can contribute to improved accuracy and spectral artifact detection. Volume holographic filters for laser intensity rejection allow simultaneous acquisition of the Stokes/anti-Stokes low-wavenumber region along with the fingerprint region of Raman spectra.

Using multivariate analysis, we demonstrate and evaluate polymorph identification from combined low-wavenumber and fingerprint Raman spectra of pharmaceutical tablet image pixels. Such spectra are found to lead to superior results compared to fingerprint spectra alone.

Keywords: Chemometrics, Pharmaceutical, Raman, Vibrational Spectroscopy
Application Code: Pharmaceutical
Methodology Code: Vibrational Spectroscopy
We have developed a new ultrafast SERS technique to study chemical reactions of molecules in plasmonic hotspots on the femtosecond timescale of molecular motion. With the first demonstration of surface-enhanced femtosecond stimulated Raman Spectroscopy1 (SE-FSRS), we have created a coherent vibrational technique capable of obtaining complete vibrational spectra with ultrafast temporal resolution. SE-FSRS combines the high plasmonic enhancements of surface-enhanced Raman spectroscopy (SERS) with the high spectral and temporal resolution of femtosecond stimulated Raman spectroscopy (FSRS). This technique is an important step towards studying chemical reactions of a small subset of molecules on the femtosecond timescale of nuclear motion. However, a number of questions remain as to how to maximize SE-FSRS signals for time-resolved studies of ultrafast reaction dynamics.

We have examined the molecule-plasmon coupling that leads to the Fano-like lineshapes observed in SE-FSRS, providing important insights into the ultrafast dynamics of molecule-plasmon coupled systems.2 Currently we are exploring a number of substrate systems to further characterize this new technique and to expand the types of molecular systems we can studying with SE-FSRS.

References:

Keywords: Raman, Surface Enhanced Raman, Ultra Fast Spectroscopy, Vibrational Spectroscopy
Application Code: Nanotechnology
Methodology Code: Vibrational Spectroscopy
The survival strategies of extremophilic organisms in terrestrially stressed locations and habitats are critically dependent upon the production of protective chemicals in response to desiccation, low wavelength radiation insolation, temperature and the presence of chemical toxins. The adaptation of life to harsh prevailing conditions involves essential control of the substratal geology; the interaction between the rock and the organisms is critical for successful colonisation and the biological modification of the geological matrix plays a significant role in the overall biological survival strategy. The identification of these biological and biogeological chemical molecular signatures in the geological record is, therefore, a crucial stage in the recognition of the presence of extinct or extant life in terrestrial and extraterrestrial scenarios. Raman spectroscopy is valuable for the detection of life extraterrestrially because of the non-invasive excitation of organic and inorganic molecules and molecular ions with high discrimination characteristics for which the vibrational spectroscopic band signatures are unique. The acquisition of experimental data without the need for chemical or mechanical pre-treatment of the specimen has been a major factor in the adoption of Raman instrumentation for future robotic planetary landers and rovers, such as the ExoMars programme. The merits of using Raman spectroscopy for the recognition of key molecular biosignatures from several terrestrial extremophile specimens will be illustrated and the technical development of a miniaturised system and its evaluation for Martian exploration is ongoing. The specimens described in this presentation have been acquired from Arctic and Antarctic cold deserts, a meteorite crater, and from hot desert saltpan evaporite locations from which the provision of key Raman spectroscopic signals have been used to detect the presence of life signatures.
**Session Title**  
Advances in Raman Spectroscopy

**Abstract Title**  
Raman Characterization of Critical Biological Reactions in Dilute Aqueous Solutions, in Single Crystals and in Living Cells

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**Abstract Text**
The beta-lactamase enzymes produced by bacteria destroy incoming antibiotics and allow the bacteria to survive. Hence much effort goes into designing inhibitors that will block the beta-lactamases. We recently developed a protocol that allows us to detect the Raman spectra of the inhibitor-enzyme reaction intermediates within live bacterial cells. These can be interpreted by Raman studies on the corresponding intermediates in dilute aqueous solution and in single crystals of the target enzymes. Taken together the data provide unique insight into the chemistry of lactamase inhibition and the design of novel compounds that may overcome the devastating consequences of drug resistance.

**Keywords:**  
Biospectroscopy, Enzyme Assays, Protein, Raman

**Application Code:**  
Drug Discovery

**Methodology Code:**  
Biospectroscopy
Western blots are useful to identify product related and host cell related proteins, but they are labor-intensive and cannot be used to quantitate proteins of interest. Recently, a new capillary based instrument was introduced that fully automates Western analysis and allows for the quantitation of proteins of interest. Applications of this new technology to vaccine development will be discussed.

Keywords: Capillary Electrophoresis
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Mitochondria require a membrane potential to produce ATP and maintain their function. Each mitochondrion appears to have a unique membrane potential, which may depend on energy demand, subcellular location, morphology, and functional status. Some of these variations may also result from disease and cellular status. It is not known how heterogeneous this subcellular property is. In this presentation we describe the use a ratiometric fluorescent probe (JC-1) and capillary electrophoresis (CE) to characterize individual mitochondrial membrane potentials. Mitochondria were isolated from cultured cells, mouse muscle or liver, labeled with JC-1, and separated with CE equipped with a post-column laser-induced fluorescence detector (CE-LIF). Distributions of individual mitochondrial membrane potential and electrophoretic mobility were observed. Analysis of specific regions of interest allowed for the examination of membrane potential and comparison of electrophoretic mobility distributions even in preparations containing depolarized mitochondria.

**Keywords:** Bioanalytical, Capillary Electrophoresis

**Application Code:** Bioanalytical

**Methodology Code:** Capillary Electrophoresis
Lipidated proteins provide a unique and unexpected challenge for characterization. The lipid itself possesses no charge but does change the hydrophobicity of the protein. One of the standard protein properties studied in characterization experiments is charge heterogeneity. Charge heterogeneity is a stability indicating property that can be monitored using capillary electrophoresis (CE). Charge heterogeneity can be measured using a variety of CE techniques such as capillary zone electrophoresis (CZE), capillary isoelectric focusing (cIEF) or imaged capillary isoelectric focusing (iCE280).

The development of a charge heterogeneity method that adequately resolves the charge variant isoforms is the goal. An IEF orthogonal method was used to gain an understanding of a lipidated protein’s molecule charge characteristics. Using this information, capillaries, separation parameters, sample preparation and capillary cleaning procedures were applied to CZE, cIEF and iCE280 to identify the best charge heterogeneity profile. Stressed samples were created to test each method. The experimental designs will be presented.

Keywords: Capillary Electrophoresis, Method Development, Protein
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Capillary Electrophoresis is a very useful tool in monitoring the production of vaccines and determining lot-to-lot consistency. Development of CE-based vaccine assays poses many challenges since vaccines are complex in nature and their size could range from 5-300 nm. This talk discusses the utility of CE-based analysis for multiple vaccine programs. CZE, MEKC, and icIEF can be used to monitor the manufacturing process, vaccine product stability, and to measure the dose of individual components in a multivalent vaccine.

**Keywords:** Biopharmaceutical, Capillary Electrophoresis, Characterization, Protein

**Application Code:** Other (Specify)

**Methodology Code:** Capillary Electrophoresis
The natural defenses of biological systems for exogenous oligonucleotides, such as synthetic antisense DNA and siRNA, present many challenges for the delivery of nucleic acids in an efficient, non-toxic, and non-immunogenic fashion. Because nucleic acids are negatively charged and prone to enzymatic degradation, researchers have relied on transfection agents such as cationic polymers, liposomes, and modified viruses to facilitate cellular entry and protect delivered biomolecules. However, each of these platforms is subject to several drawbacks, including toxicity at high concentrations, the requirement of specialty nucleic acids to enhance stability, and severe immunogenicity. Spherical nucleic acid (SNA) gold nanoparticle conjugates (inorganic nanoparticle cores functionalized with a spherical shell of densely organized, highly oriented nucleic acids) pose one possible solution to these problems in the context of antisense and RNAi pathways. Remarkably, these highly negatively charged SNA structures do not require cationic transfection agents or additional particle surface modifications and naturally enter all cell lines tested to date (over 50, including primary cells).

Further work has shown the cellular uptake of these particles to be dependent upon DNA surface density: higher densities lead to higher levels of particle uptake. The high density polyvalent nucleic acid surface layer recruits scavenger receptors from the cells that facilitate endocytosis. Methods to synthesize such structures and current cellular and animal work focused on developing them as single-entity agents for the treatment of skin disease and brain cancer will be described. SNAs harness the great promise of biotherapeutic gene silencing as a personalized medicine approach to neutralize many genes, including undruggable oncogenes, and can overcome some of the major challenges associated with CNS-directed drug delivery and RNAi-based therapy.
Precise control of nanoparticle (NP) fabrication allows for the identification of the optimal characteristics to design NPs that enhance chemotherapy and immunotherapy. The Particle Replication in Non-wetting Templates platform has been utilized to fabricate novel cross-linked hydrogel and poly(lactide-co-glycolid) (PLGA) particles with variable size, shape, drug loading, modulus, surface chemistry, targeting ligand density, and therapeutic release kinetics using triggered linkers for chemotherapeutics. In vivo, PLGA docetaxel particles with decreased particle (80 nm) diameter and drug loading (9%) had reduced drug distribution into the liver and spleen compared to particles with 200 nm diameter and 20% loading. Furthermore, decreased drug release kinetics through use of an acid-labile prodrug improved the tolerability of docetaxel, which allowed for a much higher maximum tolerated dose to enhance efficacy. Plasma circulation time of particles was controlled by surface chemistry and modulus. Higher density of poly(ethylene glycol) (PEG), as well as reduced particle cross-link density was demonstrated to extend the terminal elimination of hydrogel particles. Further studies are being conducted to optimize surface chemistry of particles with targeting ligands to enhance tumor uptake and retention. Particle Replication in Non-wetting Templates platforms may be used as a precise tool in nanobiotechnology to delineate the optimal particle design to enhance delivery of cancer therapy.

Keywords: Biopharmaceutical, Biotechnology, Drugs, Nanotechnology

Application Code: Biomedical
Methodology Code: Other (Specify)
Nanoparticle drug delivery systems afford researchers the ability to increase drug solubility, alter pharmacokinetics, target specific locations in the body, provide controlled release of a drug and thereby improve drug efficacy while limiting systemic side effects. We have developed unique pH-responsive drug-loaded expansile nanoparticles (eNPs) that localize to tumors, traffic through the lymphatic system, swell to release drug once inside the cell, and provide a depot for prolonged exposure of the cell to the drug. In this presentation, I will discuss the mechanism of action and performance of paclitaxel loaded eNPs in several cancer models including intraperitoneal mesothelioma. The synthesis of the eNPs is described first followed by several particle characterization techniques, including qNano, DLS, SEM, and TEM that measure particle size as a function of pH and swelling time. We next quantify the unique ability of drug-loaded eNPs to act as drug depots for paclitaxel within the cell as well as eNPs to enter the cell via macropinocytosis as confirmed by confocal microscopy and flow cytometry studies using temperature-sensitive metabolic reduction, pharmacologic inhibitors, and fluid-phase marker co-localization. And, finally, we demonstrate, in vivo, the improved performance of paclitaxel when delivered using the eNPs compared to cremophor/ethanol, the current standard of care, in murine mesothelioma models.

Keywords: Biomedical, Drugs, Polymers & Plastics
Application Code: Nanotechnology
Methodology Code: Fluorescence/Luminescence
Complete surgical resection of all malignant disease remains the only sure cure for cancer. To facilitate quantitative disease removal, we have developed tumor-targeted fluorescent dyes that will bind and endocytose into cancer cells and thereby ensure their distinction from adjacent healthy cells (1-3). In my presentation, I will summarize both preclinical and clinical data on the design, synthesis and use of tumor-targeted fluorescent dyes for intra-operative imaging of both human cancers and various types of inflammatory lesions. Mention will also be made of several new targeting ligands that can specifically deliver attached drugs to cancers of the colon, lung, pancreas, prostate, skin, breast, stomach, liver, and brain. I will also describe novel near infrared fluorescent dyes that excite at well beyond 800 nm where they are visible through many layers of tissue. Together, these new building blocks provide raw material for the assembly of a new generation of very bright tumor-specific fluorescent dyes for fluorescence guided surgery.

Despite the emergence of promising, new low-molecular-weight and biologic agents for cancer therapy, only a subpopulation of patients is treated effectively due to tumor heterogeneity or the development of resistance, among other factors. Nanotechnology encompasses a variety of new approaches to cancer diagnostics, imaging and therapy that may overcome some of those classical hurdles. For example, in their simplest implementation, nanoparticles can provide enhanced, selective delivery of toxic payloads to cancer while avoiding normal tissues through passive or active targeting. Through tunable pharmacokinetics and flexible, hetero- and multi-valent targeting, nanoparticles may begin to address the problem of tumor heterogeneity that confounds many agents designed to bind to a uniform target. Merged with new tools in molecular-genetic imaging and therapy, nanotechnology can lead to cancer-specific theranostic constructs. Site-specific protein engineering can be leveraged to revive agents that were once promising but failed clinically due to poor pharmacokinetics. Applications of nanotechnology to clinically important problems will be discussed, with an emphasis on agents poised for clinical translation.

Keywords: Drugs, Gene Therapy, Imaging, Nanotechnology
Application Code: Biomedical
Methodology Code: Other (Specify)
Silica-based monoliths containing nanoparticles were prepared in capillary columns for capillary liquid chromatography. Two different types of nano-particle modified monoliths were synthesized. In one, an ally-silica hybrid monolith was first prepared from silane precursors via sol-gel processing and subsequently modified with nanodimonds. The nanodimonds had been hydrogenated prior to functionalizing the capillary monolithic column. A second type of monolith was also synthesized from silane precursors but one of the precursors consisted of carbon dots modified with reactive silanes. Interestingly, the carbon dot monoliths showed a characteristic blue color under UV light radiation, which was the result of incorporating the carbon dots nanoparticles. This presentation will focus on the details of column preparation, physicochemical characteristics, and preliminary chromatographic evaluation of these new types of monoliths.

Keywords: Capillary LC, Characterization, Chromatography, HPLC Columns
Application Code: Other (Specify)
Methodology Code: Liquid Chromatography
Prospects for Organic Monoliths in Capillary Liquid Chromatography

The chromatographic efficiencies reported for organic monolithic columns for capillary LC continue to be significantly inferior to those reported for small particle packed columns. This can result from the characteristic high permeability (if due to large average through-pore diameter), broad pore-size distribution and relatively poor homogeneity of polymer monoliths. The ideal monolith structure would possess uniform through-pores similar in size to those created in very small particle packed columns, but skeletal cross sections smaller than the diameters of particles used to produce these small through-pores. A major advantage of monoliths over packed columns is the ability to independently optimize pore and skeletal sizes. Most methods investigated for synthesis of organic polymer monoliths have not provided sufficient control of pore size distribution and morphology to fully realize their full potential as stationary phases. Recent work in our laboratory using living polymerization methods have improved monolithic bed structures and resulted in chromatographic efficiencies for capillary LC in excess of 150,000 theoretical plates per meter.

Keywords: Capillary LC, HPLC, HPLC Columns, Liquid Chromatography

Application Code: General Interest

Methodology Code: Liquid Chromatography
The porous polymer monoliths emerged in the late 1980s and their first application was the separations of proteins. Since then, porous polymer monoliths found numerous other applications such as HPLC, capillary electrochromatography, gas chromatography, ion chromatography, solid phase extraction, and enzyme immobilization. Their well known advantages include ease of the preparation, robustness, high permeability to flow, mass transfer via convection, and a vast variety of chemistries. These features made them quite useful for the separation of large molecules such as proteins, synthetic polymers, and particles including nanoparticles and viruses. However, achieving good column efficiency for small molecules has been a challenge due to irregular morphology and a small surface area typical of these monoliths resulting from lack of the mesopores. We have recently developed and demonstrated the second generation monoliths using a new two-step approach to the control of porous properties that includes hypercrosslinking reaction. This technique includes the preparation of generic monoliths followed by their solvation and rapid crosslinking. This process enables the preparation of porous polymer monoliths possessing large surface areas of several hundreds m\(^2/g\). In addition to hypercross-linking of typical poly(styrene-chloromethylstyrene-divinylbenzene) monoliths, we are also exploring use of monomers with protected functionalities to introduce reactivity needed for new applications. Alternatively, we are preparing a simpler type of monoliths by copolymerization of styrene and divinylbenzene that is then hypercrosslinked using external crosslinkers such as 4,4’-bis(chloromethyl)-1,1’-biphenyl, \(\text{C}_\text{H}_\text{Cl}_\text{H}_\text{Cl}_\text{-p-xylene}\), and formaldehyde dimethyl acetal. When we used a monolith which thermally initiated polymerization was terminated after 2.5 h, the surface area of monoliths increased from 70 m\(^2/g\) to unprecedented 900 m\(^2/g\) after hypercrosslinking, i.e. thirteen times.

**Keywords:** Capillary LC, HPLC, Materials Science, Polymers & Plastics  
**Application Code:** Biomedical  
**Methodology Code:** Liquid Chromatography
Increasing Capillary HPLC Speed

The availability of higher pressure pumps and the recognition of temperature as an important variable in controlling band spreading as well as speed has led to faster separations. We are working on improving the speed of capillary LC while maintaining good concentration detection limits. This often requires injecting large volumes (relative to the column volume) in order to preconcentrate. There exists a complex set of trade-offs among sensitivity, resolution, and speed. We have shown that column diameter is an important parameter for optimization. Also, the standard preconcentration strategy of injection of a mixture in a weak solvent relies on there being a contrast in solvent strength between the chromatographic mobile phase and the injected liquid sample. The latter is usually aqueous (for reversed phase separations). In comparison to separations at room temperature, preconcentration by this method at high temperature is not as effective for modestly hydrophobic compounds because the contrast in solvent strengths is smaller. This has led us to use active temperature control to enhance preconcentration and decrease volume overload effects.
Abstract Text

Mass Spectrometry (MS) plays a number of key roles in the discovery and development phases for modern pharmaceutical compounds, ranging from the assessment of protein-ligand binding to biomarker discovery. Historically, however, MS has had a relatively limited role the drug discovery process in comparison to high-throughput fluorescence and radiometric screens. This picture may be changing, however, as many presumptive protein targets are coupled to human disease pathways through specific protein-protein interactions and protein conformations, rather than through enzyme activities. This fact will likely drive the development of high-throughput analytical tools that put a stronger emphasis on the structural information content produced in a screen.

Recently, we have been actively developing nano-electrospray ionization (nESI) coupled to ion mobility-mass spectrometry (IM-MS) into such a tool, based on its ability to rapidly separate different structures of intact multiprotein-ligand complexes from mixtures, using microgram amounts of protein and without requiring covalent labels or tagging. Our IM-MS protocols include the collision induced unfolding (CIU) of proteins and complexes, which functions as a highly sensitive and selective analog of isothermal calorimetry for gas-phase protein-ligand complexes. Here, we will present our latest IM-MS results where we demonstrate the ability of this technology to detect activation state selective inhibitors in multiple tyrosine kinases, evaluate key conformational changes within amyloid protein-ligand complexes, and identify signatures associated with cooperative ligand binding within large multiprotein assemblies.

Keywords: Drug Discovery, Electrospray, Mass Spectrometry, Protein
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
The gas-phase structure of molecules, and the relationship between gas-phase and solution-phase structure, has been explored with the assistance of ion mobility spectrometry for over 15 years, yet the lack of resolution in current instrumentation hinders more precise separation and characterization. Recently, we have obtained resolving powers over 1000 using a circular drift tube, a significant improvement over previous ion mobility drift tubes. In this talk, we will present a new instrument that has been designed with two modes: a low resolution mode using a linear drift tube and a high resolution or zoom mode via orthogonal injection into a circular drift tube. The low and high resolution modes are to be combined to enable rapid analysis of complex mixtures while also allowing for the interrogation of putatively overlapping species. Instrumentation developments for increased ion transmission in the circular drift tube and for orthogonal injection from a linear drift tube into a circular drift tube, and back, will be discussed.
Ion mobility-mass spectrometry (IM/MS) is rapidly becoming a valued tool in life science research. One advantage of IM/MS over traditional biophysical methods, in particular when studying structures and interactions of oligomeric assemblies, is the ability of IM/MS to unravel structural information on a steady-state of rapidly interconverting oligomers. Examples of biological IM/MS applications include the structural determination of toxic soluble oligomers of Alzheimer’s peptide amyloid-[beta], the elucidation of self-assembly pathways of amyloid-forming peptides, and viral capsids.

Pivotal to structure elucidation with IM/MS is a means of accurately relating the ion mobility spectrum to molecular structure. To this end, computational methods are first utilized to propose candidate structures of the compound of interest. In a second step of the methodology, collision cross sections are calculated for these candidate structures for comparison with the experimental value. The comparison between experiment and theory then reveals the detailed structure of the investigated compound. This procedure currently challenges successful application of IM/MS in the life sciences, largely because established methods cannot handle the huge size and complex shape of biological macromolecules. An additional challenge to the methodology arises from the fact that most studies currently record ion mobility data in nitrogen but compare the resulting cross sections to computational data predicted for helium as a drift gas.

Here, the use of experiment & theory to unravel structural details of large protein oligomers by IM/MS will be discussed. Specifically, the molecular mode of Phase II Alzheimer’s drug candidates on remodeling of amyloid fibers and recent results on chemokine oligomers will be presented. Furthermore, the effects of different drift gases on macromolecular structure will be discussed with respect to computational strategies used for IM/MS-based structure elucidation.
Analytical science is expected to address increasingly complex samples, primarily of biological or environmental origin, with high throughput. These demands have motivated interest in gas-phase ion mobility separations (IMS) to substitute for or complement condensed-phase (chromatographic or electrophoretic) methods prior to mass spectrometry. The more recent approach of differential or field asymmetric waveform IMS (FAIMS) is flexible and highly orthogonal to MS, but had been constrained by modest separation power. In the last few years, the FAIMS resolving power (R) was dramatically improved by use of stronger electric fields, novel gas buffers comprising helium or hydrogen, and extended filtering times. These innovations have allowed raising R up to ~500 for multiply-charged peptides and proteins, enabling previously unthinkable separations. This talk will review the emerging FAIMS applications to isotopomers, tryptic and large peptides with sequence inversions and variant localization of post-translational modifications, and protein conformers where specific geometries have apparently been distinguished. The limitations of technique for large macromolecules will be discussed.

**Keywords:** Instrumentation, Mass Spectrometry, Peptides, Protein

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
Ion Mobility Separations in Proteomics and Structural Biology

Using High Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS) to Improve Protein Discovery by Mass Spectrometry

High field asymmetric waveform ion mobility spectrometry (FAIMS) is an atmospheric pressure ion mobility technique that separates gas phase ions according to their characteristic differences in mobility in high and low electric fields. FAIMS is distinct from drift tube ion mobility spectrometry (IMS), in which the analyte ion stream is sampled in discrete packets, and ions are separated according to their size-to-charge ratio. FAIMS uses an inert carrier gas to move ions to the detector in a direction orthogonal to an electric field that alternates between a short period of one polarity at high magnitude and a long period of the opposite polarity at low magnitude. Only ions with a specific ratio of high field and low field mobility will be at equilibrium between the electrodes and reach the detector; all others will expire within the device. The waveform can be tuned to select ions of different mobility ratios.

FAIMS separation is orthogonal to both liquid chromatography (LC) and mass spectrometry (MS), and is easily coupled with electrospray ionization. We have shown that adding FAIMS to LC-MS improves protein identification by providing automated, hands-free, on-line, gas-phase fractionation. Our further developments include the modification of a commercially-available FAIMS device to significantly improve its performance for proteomics applications. We demonstrate that decreased gap LC-FAIMS-MS significantly improves identification of proteins in complex biological samples without the need for prior fractionation. We discuss the significant improvements in decreased gap FAIMS to select ions of desired charge state and charge density with the focus on selecting doubly- and triply-charged peptides, ideal for collision-induced dissociation, or high charge density peptides, ideal for electron transfer dissociation. We demonstrate significant improvements in protein identification and quantification.

**Keywords:** Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry, Proteomics

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

**Methodology Code:** Mass Spectrometry
In situ mass spectrometry in extreme environments, such as in the deep ocean or outer space, poses significant challenges. Many of these challenges are common, including the requirements for high reliability, autonomous or remote operation, ruggedness to deal with rapidly changing environmental conditions, and restrictions on size, weight, and power. Other engineering concerns are specific to the particular environment, such as extreme pressure differentials between ambient hydrostatic pressure and mass spectrometer vacuum at depth in the ocean, and extreme temperature ranges in deep space.

We have been developing and using underwater mass spectrometers for over 15 years. To date, all instruments have been based on membrane introduction mass spectrometry (MIMS). We have deployed these MIMS instruments on a variety of underwater platforms, such as deep and shallow tow sleds, vertical profiling frames, remotely operated vehicles (ROVs), and autonomous underwater vehicles (AUVs). One of the primary targets in recent years has been in situ characterization of underwater natural hydrocarbon seeps and leaks.

More recently, we have begun to design and test a prototype micro-machined cylindrical ion trap array ([micro]-CIT) mass spectrometer aimed at detecting and identifying low-molecular-weight biomarkers, ultimately in space missions of astrobiological relevance. We plan to demonstrate that the [micro]-MS can measure molecules up to 250 atomic mass units, allowing us to detect and characterize water and compounds of prebiotic relevance, such as those on the surface of comets. This effort involves adapting and improving previous [micro]-CIT array designs developed over the last ten years.

Funding acknowledged from NASA Contract No. NNX12AQ26G.
Mass spectrometry, potentially combined with gas chromatography, is widely considered the “gold standard” for chemical detection, identification and quantification. A truly portable mass spectrometer would have numerous applications in a variety of areas ranging from border protection, airport security, first responder protection, and forensics and for the detection of environmental hazards etc. Developers of such systems have all come to recognize that one of the main obstacles to truly portable mass spectrometry is the size, weight and power consumption of the necessary vacuum pumping system.

For a number of years, we has been developing small, lightweight and low power pumping systems for NASA and ESA planetary missions including two pumps now on the Curiosity rover on Mars. For these planetary missions the major requirements are high reliability, predictable lifetime and unattended operation as well as the ability to operate in an extremely harsh environment over a wide range of temperatures. Consequently, the pumps are largely hand-built custom designs that have been thoroughly tested and qualified, whereas low-cost, mass production capability has not been a key design driver.

Recently, we have conducted a number of projects to design lower cost pumping systems that are targeted for terrestrial applications. The resulting pumping system includes a scroll roughing pump mated with either a molecular drag pump for vacuum levels around 0.1 mtorr or a turbo molecular pump capable of 0.1 microtorr or better. The system also includes a single board microcontroller based electronic drive. Total weight of the final system will be less than 700 gram and total power consumption less than 10 W continuous.

The paper will describe current development program status and future plans for pumping system development as well as system test results.

**Keywords:** Gas Chromatography/Mass Spectrometry, GC-MS, Mass Spectrometry

**Application Code:** Drug Discovery

**Methodology Code:** Mass Spectrometry
In this presentation we will discuss the concepts for designing different types of mass spectrometry (MS) devices for point-of-care (POC) applications including quantitation of biomarkers in biofluids, profiling of tissues and endoscopic chemical analysis. The new designs leverage previous development of Mini 12 mass analysis system, which was a benchtop miniature instrument with paper spray sample cartridge for POC analysis. Mini 12 and its predecessors developed at Purdue use the discontinuous atmospheric pressure interface (DAPI) to allow the coupling with atmospheric and ambient ionization sources using miniature pumping system. Scan time of 1s or longer is typically required for DAPI-MS systems, which are significantly reduced with the implementation of multiple MS/MS scans for each sample/ion introduction. Methods for direct sampling of tissue using desorption ionization and transferring ions over long distance have been developed and used for design of an endoscopic probe. The desorption ionization has been achieved without high electric voltage or organic solvent, which are not compatible for in vivo analysis. Efficient ion transfer has been achieved and good quality spectra were obtained for lipids using 1/16 in ID Tygon tube of meters long. New pumping technologies are also tested in different configurations for DAPI-MS instruments, which provide insights for the future instrumentation of MS-based POC systems.

Keywords: Bioanalytical, Biomedical, Mass Spectrometry
Application Code: Biomedical
Methodology Code: Mass Spectrometry
Conventional single quadrupole electrospray ionization mass spectrometers are bulky, cumbersome, and inflexible. They are consequently often operated as a dedicated central facility rather than as part of a chemist’s everyday toolkit. The need for a more deployable, general purpose detector for the laboratory has been met by the commercial development of a miniature instrument. Microelectromechanical systems (MEMS) techniques have been employed to develop an electrospray source, a vacuum interface, a quadrupole ion guide, and a quadrupole mass filter. The evolution of these components, and the concepts underpinning their design will be described. The complete instrument, including the backing pumps and computer, is entirely contained within a single enclosure. It can be conveniently coupled to flash and analytical liquid chromatography systems or used for continuous flow reaction monitoring. These and other applications will be discussed.

Keywords: Electrospray, HPLC Detection, Mass Spectrometry, Quadrupole MS
Application Code: Other (Specify)
Methodology Code: Mass Spectrometry
Recent technological developments have enabled the transition of mass spectrometry (MS)—traditionally a highly technical, laboratory-based detection technique—into an important instrument for security applications. This evolution is driven by the development of new classes of MS instrumentation capable of confirmatory analysis with minimal sample preparation and ruggedized for field-based operation. A key driver for MS adoption is the inherent selectivity afforded by MS, leading to high fidelity target identification and confirmation.

Historically, market acceptance of mass spectrometry for security applications has been limited because of size, power, and cost considerations. Adoption of MS is highly dependent on the introduction of systems that meet the requirements for outside-the-lab use, including the system’s ability to accept samples with minimal sample preparation. Additionally, simultaneous measurement and alarming is needed, and has been achieved to a degree, through the implementation of more robust real-time, data-searching techniques. Fielding these advancements in circumstances such as incident response, HAZMAT identification, building force protection, and others, while maintaining a field-viable form factor (in terms of size, weight, and power), represents a significant R&D effort by the MS community.

A further consideration of fielded systems is the technical ability of the end user. Operators in many security applications are generally not MS experts, therefore requiring the adaptation of MS technology into familiar concepts of operation is critical for broader security market acceptance.

This presentation will focus primarily on the implementation of MS technology in multiple security uses, with an overview of the history of adoption of mass spectrometers for security and defense applications.

Keywords: Forensics, GC-MS, Mass Spectrometry, Portable Instruments
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
Carbon nanotube networks represent a powerful platform for the generation of chemiresistive sensors. Through the selective chemical functionalization with selector molecules and/or receptors, the conductance can be made selective to the presence of analytes ranging from large biological macromolecules to small gaseous molecules. This lecture will detail the fabrication of robust sensors using new functionalization chemistry to impart selectivity to the carbon nanotubes and methods to minimize drift that is often inherent in resistivity-based sensing schemes. Sensor arrays with low levels of cross-reactivity between sensor elements will be discussed for the robust classification of chemicals. The use of carbon nanotube-based gas sensors for the detection of ethylene and other gases relevant to the monitoring fruit and produce ripeness will be presented. Gas sensors for agricultural and food production/storage/transportation are being specifically targeted and can be used to create systems that increase production, manage inventories, and minimize losses.
The analysis of carbon dioxide (CO2) provides valuable information on health and environmental applications. In this work, we have developed, and characterized a pocket-sized CO2 analyzer for real-time analysis of end-tidal CO2, and environmental CO2. The CO2 sensor shows fast and reversible response to CO2 levels from few part-per-million (ppm) to 11.5%, including with the environmental and health applications. A cross-interference study is performed, which indicates that the CO2 sensor is not sensitive to the interfering gases in ambient or expired air. Furthermore, the results assessed with the analyzer in presence of real breath are in good agreement with those from a commercial infrared analyzer.
A concept of chemical sensors with adjustable affinity will be discussed. Such a device being a further development of conductometric signal transducing was realized on the base of conducting polymers. The six-electrode consists of four measurement electrodes providing simultaneous monitoring of polymer and contact resistance and two additional electrodes (auxiliary and reference) to control redox-state of the polymer. An integration of all six electrodes into one chip allows one to apply this device not only in conducting environment but also in gases and in other non-conducting media. In comparison with usual conductometric sensors, this configuration, named as integrated electrochemical transistor, provides a number of additional features, such as: (i) electrical control of sensor affinity; (ii) electrically driven fast regeneration after exposure to oxidizing or reducing analytes; (iii) uncoupling of redox- and pH-effects and development of ultrasensitive pH-sensors. First examples of application of this new technology include a fast and reversible sensor for nitrogen dioxide, an ultrasensitive pH-indicator and a sensor for QC of sea products.
We have explored all-electronic chemical detectors based on bio-nano hybrids, where the biomolecule (protein or DNA) provides chemical recognition and a carbon nanotube (NT) or graphene transistor enables electronic readout. This sensor class represents a promising approach towards sensitive detection of liquid- and vapor-phase analytes. NT or graphene transistors are functionalized with proteins through an amide bond using a robust process based on carboxylated diazonium salts. Control of protein orientation is achieved through a variety of approaches that provide affinity for specific sites on an engineered protein, e.g., a histidine tag or cysteine residue. We used this approach to create nanoelectronic interfaces to a variety of different proteins including: antibodies and antibody fragments, G-protein coupled receptors (GPCRs) housed in nanoscale membrane analogues, and GPCR variants that were engineered for enhanced solubility and stability in aqueous solution. We demonstrated the use of these bio-nano hybrids for detection of protein cancer biomarkers, antigen from various pathogens, and small molecule receptor targets at concentrations ~ 1 pg/mL. Non-covalent functionalization is achieved by self-assembling monolayers of single-stranded DNA on the graphene or NT sidewall. The DNA is used for its chemical recognition for small molecule analytes rather than recognition of complementary DNA. Vapor sensors based on this approach were able to discriminate between highly similar compounds such as enantiomers, and they show chemical responses that vary with the based sequence of the DNA, making this a promising pathway towards a system for machine olfaction. The work was supported by the Nano/Bio Interface Center (an NSF-funded NSEC), Lockheed Martin, the Air Force Research Laboratory, Intel, and DARPA.

Keywords: Nanotechnology, Semiconductor, Sensors, Biosensors
Application Code: Nanotechnology
Methodology Code: Sensors
Our team is developing new concepts of chemical and biological detection with multivariable sensors, where an individual sensor has several partially or fully independent responses. Such single sensor has several advantages over sensor arrays that include reduced number of noise sources, more predictable sensor aging, and simplified fabrication/packaging. In our talk, we will discuss our examples of such developed sensors that include electrical and mechanical resonators and optical sensors based on nanostructures, core-shell nanoparticles, and plasmonic nanocomposites.
The first attempts to analyze peptides by mass spectrometry date back to around 1960. At that time the only ionization method was electron impact ionization which required volatile samples and as a consequence extensive derivatization of the peptides. In the 1970’s two new ionization methods, FD and CI were tested but without any real improvement in spite of the fact that FD allowed analysis of native peptides. The first real breakthrough came in the early 1980’s with the advent of the soft ionization methods PDMS and FAB which both allowed analysis of small to medium sized proteins without prior derivatization. A number of the concepts presently used in proteomics were developed based on these two methods in spite of the fact that they both suffered from limitations in mass range as well as sensitivity to reach the needs of biological research. In this period the concept of tandem mass spectrometry was introduced as a way to generate sequence information of the pseudo molecular ions generated by the soft ionization methods. Hillenkamp and Karas discovered MALDI in the mid 1980’s and really demonstrated its potential for protein analysis in 1988. In the same year Fenn demonstrated the capability of ESI for such analysis. Independently of Hillenkamp-Karas’ discovery, Koichi Tanaka a little later demonstrated that laser desorption allowed analysis of intact proteins by dissolving proteins in a glycerol matrix containing fine metal powder. My lecture will describe the history of the pioneering period in peptide and protein mass spectrometry up to the successful introduction of MALDI and ESI.

Keywords: Bioanalytical, Mass Spectrometry, Peptides, Protein
Application Code: Genomics, Proteomics and Other ’Omics
Methodology Code: Mass Spectrometry
All modern TOF instruments are based on the pioneering work of Wiley and McLaren sixty years ago with the addition of the two-stage ion mirror by Mamygin nearly fifty years ago. Prior to the invention of MALDI, instruments were developed and applied to a variety of research applications and some minor improvements in performance were realized. The invention of electrospray by Fenn and MALDI by Karas and Hillenkamp opened mass spectrometry to a wide variety of biological applications. Applications of electrospray developed rapidly using established MS analyzers such as quadrupoles and ion traps, but MALDI was most compatible with TOF that was not highly developed nor widely used. The potential power of MALDI for many important applications has fueled rapid development of TOF techniques and very high performance instruments are now available from several vendors. The present status of TOF instrumentation for biological applications will be reviewed and summarized.

Keywords: Biotechnology, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
MALDI Imaging MS produces molecular maps of peptides, proteins, lipids and metabolites present in intact tissue sections. It employs desorption of molecules by direct laser irradiation to map the location of specific molecules from fresh frozen and formalin fixed tissue sections without the need of target specific reagents such as antibodies. Molecular images are produced in specific m/z (mass-to-charge) values, or ranges of values, typically covering the MW range 200-100,000. We have developed histology-directed molecular analysis, whereby only selected areas of cells in tissue are ablated and analyzed.

We use Imaging MS in a variety of biologically and medically relevant research projects. An area of special interest is molecular mapping of changes observed in diabetes in both a mouse model and in human disease. Major molecular alterations have been recorded in advanced diabetic nephropathy. Other applications include developmental studies of embryo implantation in mouse, renal cancer and other organs, and neurodegenerative disease. Molecular signatures have been identified that are differentially expressed in diseased compared to normal tissue and also in differentiating different stages of disease. These signatures typically consist of 10-20 or more different proteins and peptides, each identified using classical proteomics methods. In addition, Imaging MS has been applied to drug targeting and metabolic studies both in specific organs and also intact whole animal sections following drug administration.

This presentation focuses on biological and clinical applications and on technological advances both in sample preparation and instrumental performance to achieve images at high spatial resolution (1-10 microns). Finally, new biocomputational approaches will be discussed that deal with data (image) processing and our implementation of ‘image fusion’ in terms of predictive integration of MS images with microscopy and other imaging modalities.

Keywords: Mass Spectrometry
Application Code: Biomedical
Methodology Code: Mass Spectrometry
Biomarker development requires the implementation of progressively standardized and increasingly rigorous analytical technologies. Regarding proteins, such technologies must be; 1) Highly accurate, sensitive and reproducible, 2) Responsive to large concentration differences and disease-specific qualitative variations, and, 3) Employed at rates sufficient to economically accommodate large clinical sample sets. Here, we present one such technology, mass spectrometric immunoassay (MSIA) and illustrate its use in the development of multi-analyte biosignatures of type 2 diabetes and related cardiovascular diseases.
Recent advances in Ultra High Performance Liquid Chromatography have focused on the development of stationary phases that allow higher resolution and/or faster separations. Coupled with Mass Spectrometry, UHPLC is the method of choice in modern LC-MS, since it provides selectivity and sensitivity in a robust format, with throughput capability that can match increasing analytical demands.

One variant that has received a lot of attention over the past years is micro-LC-MS, using columns of 1 mm ID or less. The interest in micro-LC-MS has been renewed due to the introduction of improved commercial hardware by LC vendors that can support this technique. Using modern split-less micro-LC fluid delivery systems, and optimized micro-LC-MS interfaces, current micro-LC-MS systems can realize the benefits of advanced particle technologies, in addition to the inherent speed, sensitivity and cost benefits of micro-LC.

We will discuss some of the fundamental advantages and engineering considerations for micro-LC, and provide examples of pharmaceutical and food applications where microLC-MS offers significant analytical advantages over conventional approaches.

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**Keywords:** Capillary LC, Chromatography, HPLC Columns, Liquid Chromatography/Mass Spectroscopy

**Application Code:** Bioanalytical

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Advances in chromatographic packing materials and instrumentation have led to the use of smaller particles packed into smaller diameter (2.1 and 3.0 mm) columns to achieve faster and more efficient separations. In order to realize the full benefit of these columns, it is critical to understand the role that extra-column dispersion plays to provide the highest separation performance per unit time. In addition, new generations of solid-core particles have allowed unprecedented increases in throughput, thus expanding the number of applications possible in all areas of separations science.

In this presentation, we will briefly discuss the impact of LC instrumentation on the performance of these new sub-2-\[\text{micro}\]m, solid-core packing materials. A majority of the presentation will be spent on the types of applications that benefit from these advances, most notably in drug discovery and development, bioanalysis, food and environmental applications, and forensic toxicology.

**Keywords:** Environmental/Water, Forensics, Liquid Chromatography, Pharmaceutical

**Application Code:** Other (Specify)

**Methodology Code:** Liquid Chromatography
Over the past several years the performance advantages of superficially porous particles (SPP) for HPLC separations have become well documented, and such materials have become commercially available from several sources. Following our pioneering efforts to document the advantages of the prototypical SPP, which we call Halo Fused-Core particles, we have undertaken efforts to define high performance Halo Fused-Core materials for larger molecule applications by taking advantage of the fast mass transfer properties of thin-shell SPP packing materials. Particularly, recent development efforts have been on materials and methods for biomolecules, including proteins, peptides, glycans, and particularly on the study of post translation modifications of proteins. Particular attention has focused on glycosylation variants of proteins, as well as fragments thereof. Selection of optimization variables has included several particle features, including particle size, shell thickness, pore size and bonded-phase characteristics, as well as selection of mobile phase conditions that favor high performance mass spectral analyses. This presentation centers on examples of high performance separations of biomolecules, demonstrating design features of modern SPP materials that permit very high resolution of complex biomolecule mixtures, using conditions that permit detailed structure information, as can be obtained from online coupling with high performance mass spectrometry.

Keywords: Biopharmaceutical, HPLC Columns, Mass Spectrometry, Protein
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Technological Advances in Ultra High Performance Liquid Chromatography

Core-shell Contributions to Particle Miniaturization in Ultra-High Performance Liquid Chromatography

The pursuit to create ultra-high performance liquid chromatography columns has taken two paths. One of these paths is the miniaturization of the particles used to pack the columns. The second path has been the use of core-shell materials, which have a solid non-porous core, and an outer porous shell. The miniaturization of particles provides an improvement in column performance that is inversely proportional to the change in particle size. By switching from a fully porous particle to a core-shell particle of the same size, one typically sees 30-50% more efficiency for the core-shell particle over the fully porous particle. In 2009 a combination of particle miniaturization and core-shell technology was introduced, providing the most efficient chromatographic columns at the time. In 2013 a further miniaturization of core-shell particles, down to 1.3 µm in size, was demonstrated, representing the next generation of improvement (approximately double the number of theoretical plates) over fully porous sub-2 µm materials. In this paper we will explore core-shell contributions to chromatographic efficiency over their fully porous counterparts. One key area of interest will be differences in frictional heating in core-shell columns versus fully porous columns and its impact on chromatographic performance. We will also explore if certain column architectures are more susceptible to frictional heating induced chromatographic degradation than others with both fully porous and core-shell materials.

Keywords:  High Throughput Chemical Analysis, HPLC, HPLC Columns, Liquid Chromatography
Application Code: High-Throughput Chemical Analysis
Methodology Code: Liquid Chromatography
The last ten years witnessed the increasing adoption of Ultrahigh Performance Liquid Chromatography (UHPLC) since the first commercial UHPLC instrument was introduced in 2004. We also saw major advancement in column technologies, most notably sub-2-μm totally porous particles and superficially porous particles. The combination of UHPLC instrument and novel particle technologies enabled chromatographers to achieve faster and more efficient separations. This certainly addresses the efficiency part of the resolution equation. For practitioners, it is also important to get good selectivity and being able to apply the new instrument/column technologies in different separation modes. In this presentation, we discuss new advances in column phase chemistries and how they can be used in different modes of separation to solve challenging problems, including new reversed-phase columns, HILIC phases, bioanalytical columns as well as SFC columns.

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### Abstract

Ten years since the commercial introduction of UHPLC instruments, application and implementation of UHPLC is still on the rise in the pharmaceutical industry. While UHPLC has not yet supplanted low pressure HPLC as the tool of choice, the growth of UHPLC in quality control laboratories and manufacturing environments demonstrates increasing confidence in using UHPLC in regulated environments. The broader acceptance of UHPLC is a direct result of improvements in instrument and column ruggedness, expanded lines of stationary phases and column geometries, and software tools to assist with method translation between HPLC and UHPLC.

While UHPLC applications for pharmaceutical analysis continue to increase, alternatives for high efficiency and fast separations have emerged as alternatives to UHPLC. Semi-porous particle technology, 2D HPLC, and on-line HPLC all offer lower pressure alternatives to increasing separation efficiency, speed of analysis and higher sample throughput. The focus of this presentation will highlight the benefits UHPLC for pharmaceutical method development and highlight the complementary nature of the aforementioned alternatives when combined with UHPLC.

**Keywords:** HPLC, Pharmaceutical, Process Analytical Chemistry

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography
Paper is a very interesting matrix for electrochemistry. The hydrophilic fibers of the paper hold aqueous solutions in place and prevent large-scale convective movement, but the cellulose does not dissolve in the water. This talk will briefly describe some of the opportunities for electrochemistry of aqueous solutions held in paper matrices, with two emphases: the development of a standard reference electrode based on paper, and exploration (in collaboration with the group of P. Buhlmann at the U. Minnesota) of the practicality of making ion-selective paper electrodes.
Inkjet-Printed Paper-Based Colorimetric Sensor Array for the Discrimination of Volatile Amines

Gas sensor arrays operating according to the “electronic nose concept”, which in analogy to human olfaction rely on cross-responsive rather than highly selective sensors, are of high interest for various fields of applications.

Here, we present a colorimetric sensor array for the discrimination of volatile amines obtained by combining two simple functional elements: (1) a single chromogenic sensing dye with selectivity for amines in general, encapsulated into (2) polymer nanoparticles of different polarities. The particles are composed either of poly(benzyl methacrylate) (pBzMA) as the more hydrophobic component or of poly(diethylene glycol methyl ether methacrylate-co-methyl methacrylate) p(DEGMA-co-MMA) as the more hydrophilic component. In order to achieve reproducible and cost efficient sensor array fabrication, inkjet-printing technology combined with standard copy paper as sensor substrate has been applied. Printing of 6 types of inks incorporating two dye encapsulating nanoparticles of different polarity in different mixture ratios, results in a colorimetric sensor array with a polarity gradient. The color differences of the sensor array spots before and 20 min after exposure to 7 primary amines of different alkyl chain substituents in the gas phase (see attached figure) were analyzed by principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) analysis. Results indicated high discrimination ability with high reproducibility for triplicate measurements down to amine concentrations of 50 ppm. The discrimination ability was maintained over a wide range of relative humidity (10 - 80 %), demonstrating the high selectivity of the array for gaseous amines. Furthermore, no interference from common volatile organic compounds was observed.

This is to the best of our knowledge the first report of a colorimetric sensor array with selectivity for a specific chemical class of analytes and the ability to discriminate compounds of the same class, which is obtained by simply mixing two types of single dye-encapsulating polymer nanoparticles.
Flow injection analysis (FIA) is a commonly used analytical method that exhibits high sample throughput capability and enhanced reproducibility compared to manual methods. Sample volumes and reagent consumption can also be reduced in FIA systems [1]. In this presentation, the possibility of using fully reversible polyanion sensitive pulstrode type membrane electrodes as detectors in FIA systems is examined. Pulstrode polyanion electrodes have been used previously in the manual mode to monitor enzymatic reactions [2] and to detect various polyanions and polycationic species [3,4].

The new FIA-pulstrode system can be used to monitor enzymatic reactions and determine heparin concentrations via titration with protamine. The detection electrode is comprised of a plasticized polymeric membrane doped with 10 wt% of tridodecylmethylammonium-dinonylnaphthalene sulfonate ion-exchanger salt. The pulse sequence used involves a short galvanostatic pulse, an open-circuit pulse, and then a potentiostatic pulse [3,4]. It will be shown that total pulse sequence times can be optimized to yield reproducible real-time detection of injected samples of protamine, heparin, and other polyanions at 10-20 samples/h, and that the same membrane detector can be employed for FIA of both polycations and polyanions at levels greater than or equal to 10 [micro]g/ml simply by changing the direction of the galvanostatic pulse.

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Keywords: Bioanalytical, Biological Samples, Electrochemistry, Ion Selective Electrodes
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Nanomaterials in Ion-Selective Sensors

Nanomaterials can offer new solutions to ion-selective measurements, either due to particular energy levels associated with the nano-dimensions, or through the diffusion and response timescales associated with their size. We have looked at extending the field through incorporation of enzyme-linked analytical nanosphere sensors (ANSors). Both optical and electrochemical systems are considered. For example enzyme-substrate turnover at a quantum dot (QD) due to co-immobilised enzyme and pH sensitive ligand or QD modified electrodes mediating electron transfer. QD-CaR (calcium red) absorption shows spectral overlap with QD530 emission at all pHs, and gives a complex pH dependent FRET efficiency, due to excited state proton transfer. In contrast QD615-CaR with spectral overlap between the QD and CaR gave a strong and reproducible pH response. QD-urease and QD-creatinine deiminase conjugates could be linked with pH changes produced by enzyme degradation of urea and creatinine respectively. In contrast, when quantum dots, were deposited on electrodes, the redox current due to certain couples was modulated. For example, direct interaction between the QDs and ferrocene dicarboxylic acid was confirmed by irreversible quenching of the fluorescence with Perrin-type plots of the quenching consistent with a static mechanism. A current rectification mechanism is examined, dependent on the overlap of the density of states between the FDCA/FDCA+ and the semi-conductor band edges and/or surface states. Using the MPA-QD at an electrode together with an immobilised glucose oxidase layer in the presence of FDCA and glucose, catalytic oxidation currents were recorded consistent with a mediation mechanism. Calculation of an apparent $k_f$ for the reaction between FDCA and the electrode showed an increase of about 30 times in the presence of QDs compared with the unmodified electrode and gave a current response due to glucose that was approximately twice that without QDs.

Keywords: Bioanalytical, Biosensors, Ion Selective Electrodes, Membrane
Application Code: Nanotechnology
Methodology Code: Sensors
Volammetric Ion-Selective Electrodes for Ultratrace Analysis

In this presentation, voltammetric ion selective electrodes with nanomolar and subnanomolar detection limits will be discussed. The sensitive detection of ubiquitous ions renders this voltammetric method highly attractive as a replacement for more expensive mass-pectrometric methods in ultratrace analysis. The voltammetric principle demonstrates that the extremely low detection limits can be ultimately limited by the contamination of background water with the ubiquitous ions. This important possibility has been unnoticed for the characterization of the potentiometric counterpart, which does not provide much information about background.

**Keywords:** Electrochemistry, Electrodes, Sensors

**Application Code:** Environmental

**Methodology Code:** Electrochemistry
Magnesium and its alloys exhibit properties such as strength, light weight, and corrosion in aqueous environments 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 of magnesium and its alloys by measuring the concentrations of the solution soluble corrosion products magnesium ion, hydroxyl ion and hydrogen gas. Potentiometric sensors for $\text{Mg}^{2+}$, pH and $\text{H}_2$, an amperometric sensor for $\text{H}_2$, and a conductimetric sensor for osmolality have been used to monitor the corrosion of magnesium in real-time during standard immersion tests for alloy corrosion as well as for $\text{i}n\text{ v}i\text{t}r\text{o}$ and $\text{i}n\text{ v}i\text{v}o$ measurements to determine the effect of the degradation products on cells surrounding an implant.
Introduction: Imaging intracellular ion concentrations is difficult for sodium and potassium, and commercially-available ion indicators have several limitations – poor signal-to-noise, sub-optimal imaging wavelengths, poor selectivity, and rapid photobleaching. Optodes offer an attractive platform for designing ion-selective nanosensors because their ion-specificity and sensitivity are based on the choice of ionophore and formulation, respectively. However, achieving nanosensors sufficiently small (<200 nm) for intracellular use and with the photostability of quantum dots has been extremely difficult. Herein, we describe the fabrication and characterization of an ionophore-based potassium-selective nanosensors that meet these important criteria as well as the necessary sensitivity and selectivity for use in mammalian cells.

Results: The nanosensors are an oily droplet of dioctyl sebacate that contains the potassium-selective optode components and quantum dots that emit at either 540 or 650 nm. The nanosensors are stabilized by a PEGylated phospholipid, DSPE-mPEG(550). After fabrication by sonication and filtering with a 100 nm syringe filter, the resulting nanosensors have a mean hydrodynamic radius of 66 ± 1 nm and a half-width of 19 ± 2 nm. This represents a size-reduction of nearly 50% from our previous generation of nanosensors. The ratiometric response to potassium has a Kd of 36 mM in media with no background interfering ions, and 45 mM with 50 mM background sodium. The sodium response has a Kd of 1200 mM, which tunes out physiological sodium dynamics. This combination of size, selectivity, and photostability is unprecedented in the literature and we are now developing intracellular potassium imaging protocols.

Keywords: Biological Samples, Fluorescence, Nanotechnology, Sensors
Application Code: Nanotechnology
Methodology Code: Sensors
Ion diffusion through the membrane influences not only achievable detection limits and selectivities, but also generally the performance of the sensor (e.g. slope, reversibility of responses) and electrical resistance of the membrane phase; thus influencing also electrochemical responses tuning possibility. Generally ion diffusion coefficients in the ion-selective membranes are at least two orders of magnitude lower compared to those in solution. Slow (in terms of electrolyte solutions) diffusion of ions in the membrane results in different effects, among others relatively long time required to achieve full membrane saturation with primary ions, on the other hand, on the electrolyte/membrane interface significant accumulation of ions can occur. These effects are increasing in significance with lowering the diffusion coefficient of ions within the membrane, i.e. when traditional poly(vinyl chloride) membranes are replaced with polyacrylate counterparts.

Accumulation of ions at the outer layer of the ion-selective membrane leads to impaired responses of ions sensors. Depending on sensor, and transport of primary ions within the membrane either non-Nernstian responses [1] or decreased selectivity is observed [2]. On the other hand, these effects can be beneficial for optical sensors based on optrode concept, e.g. fluorimetric sensors. In this case accumulation of analyte ions in the surface region of sensor can lead to pronounced increase of linear responses range to cover seven orders of magnitude, as observed for Zn2+ ions fluorimetric sensor [3].


Keywords: Ion Selective Electrodes, Potentiometry, Sensors
Application Code: Biomedical
Methodology Code: Electrochemistry
Abstract Text
We present the development of a reporter-probe displacement biosensor for applications in two-photon microscopy. The speed, selectivity, sensitivity, and extent of false positives for this biosensor were investigated. In general, current biosensor technology relies on disrupting a quenching mechanism to generate signal. Unfortunately this approach will give rise to false positives if the biosensor is used in tissue or cellular applications because of nuclease degradation. We have designed an innovative biosensor that brings Cy5 and a quencher within proximity to allow for quenching of the fluorescence. In this way false positives can be rejected because the dyes must be forced together to create a change in analytical signal. The reporter-probe complex can selectively bind to miRNA targets within 15 minutes and reduces false positive signals from nuclease degradation by at least 20 % compared to molecular beacons. The limits of detection using both Cy3 and Iowa Black quencher were investigated.

Keywords:  Bioanalytical, Biological Samples, Biosensors, Biospectroscopy
Application Code:  Bioanalytical
Methodology Code:  Biospectroscopy
Sepsis is a blood infection that has a high incidence of mortality. Early diagnosis and treatment of sepsis would benefit enormously from systems that could provide rapid and active intervention to identify and remove blood borne agents. The ability to rapidly detect the molecular and pathogenic markers for sepsis requires the development of new sensing technologies that are rapid, do not require labels or extended processing of the sample, detect multiple targets simultaneously, and are sufficiently stable towards fouling to permit continuous sensing over clinically relevant timescales. Sensors based on surface-enhanced Raman spectroscopy (SERS) are ideal because they can provide ultrasensitive detection of molecular analytes without the need for labels and sample processing and with excellent discrimination against background analytes. We will present results on the development of a SERS-based sensor for real-time monitoring of analytes in blood, including bacteria and glucose. SERS sensors offer a new opportunity to perform rapid, label-free, and ultrasensitive analysis of molecular analytes in blood and other complex samples.

Additionally, we will discuss progress made using super resolution plasmon microscopy (SRPM), which is a flexible technique for label-free biosensing and sub-diffraction limited imaging that uses the plasmonic properties of single nanoparticles. Using SPRM, we are able to simultaneously track the spatial position and LSPR spectra of individual gold nanoparticles diffusing across a cell membrane mimetic (supported lipid bilayers) containing a ganglioside lipid (GM1). At present the characteristics of SRPM are: (1) spatial resolution = 6.7 nm, (2) time solution = 33 msec, and spectral resolution = 1 nm.

Keywords: Bioanalytical, Biosensors, Surface Enhanced Raman
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Nature and Nanotechnology likewise employ nanoscale machines that self-assemble into structures of complex architecture and functionality. Fluorescence microscopy offers a non-invasive tool to probe and ultimately dissect and control these nanoassemblies in real-time. In particular, single molecule fluorescence resonance energy transfer (smFRET) allows us to measure distances at the 2-8 nm scale, whereas complementary super-resolution localization techniques based on Gaussian fitting of imaged point spread functions (PSFs) measure distances in the 10 nm and longer range. Here, I will describe a method for the intracellular single molecule, high-resolution localization and counting (iSHiRLoC) of microRNAs (miRNAs), a large group of gene silencers with profound roles in our body, from stem cell development to cancer. Microinjected, singly-fluorophore labeled, functional miRNAs are tracked as individual diffusing particles inside single human cells. Observed mobility and mRNA dependent assembly changes suggest the existence of two kinetically distinct assembly/disassembly processes, thus bringing into focus a unifying molecular mechanism for the ubiquitous RNA silencing pathway.
### Session Title
Spectrochemical Analysis of Biological Systems - A Perspective from New and Established Investigators

### Abstract Title
Examination of UV-Excited Fluorescence and Resonance Raman Spectroscopy for Determination of DNA/ Protein Ratios

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**Abstract Text**

The histopathological diagnostic value of intracellular DNA / protein ratios is well established, but autofluorescence and the chemical complexity of tissue make label-free in vivo or in vitro measurement of DNA and protein concentrations via visible-wavelength or near infrared Raman spectroscopy challenging. Ultraviolet (UV) excitation provides an intriguing alternative due to strong absorption by DNA and aromatic amino acids, and because Raman scattering and fluorescence are spectrally separated when molecules are excited in the deep UV. Due to these two unique strengths, our laboratory is investigating the use of UV resonance Raman spectroscopy and UV-excited fluorescence for rapid, label-free determination of DNA / protein ratios. We will discuss the strengths and weaknesses of deep UV excitation, as well as preliminary results.

**Keywords:** Bioanalytical, Biomedical, Raman

**Application Code:** Bioanalytical

**Methodology Code:** Molecular Spectroscopy
Developing a Diverse Toolkit for Detecting and Treating Epithelial Ovarian Cancer

Characterized by late clinical presentation, significant co-morbidity and poor long-term survival, epithelial ovarian cancer (EOC) remains a serious medical concern to women. In the Whelan lab, we leverage a diverse set of tools drawn from bioanalysis, spectrochemistry, nanoscience, molecular biology, and bioinformatics to develop new detection methods and theranostic approaches for EOC. We have used capillary electrophoresis with laser-induced fluorescence detection to select new DNA aptamers for several ovarian cancer biomarkers. Aptamers have also been selected using ovarian cancer cell lines with differential biomarker expression as positive and negative targets, to develop aptamers with high affinity for ovarian tumor cells with high metastatic potential. High-throughput sequence data generated at each stage of the selection process has opened new insights on the survival and enrichment of candidate aptamers and, when mined with data analysis tools adapted from genomics research, enable in silico characterization of sequence and structure to complement wet lab methods. These aptamers are being used as the basis of simple, “naked eye” colorimetric assays for cancer biomarkers in serum and as cell-targeting vehicles for thermal therapy and in vivo imaging. We acknowledge support from the National Cancer Institute.

Keywords: Bioanalytical, Capillary Electrophoresis, Nanotechnology, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Biospectroscopy
The combination of therapeutics and diagnostics into a single construct, i.e. theranostics, is an emerging field of medical research that aims to further improve personalized medicine in a seamless fashion. This lecture provides an overview of recent developments in our laboratory for several plasmonic nanomaterials and biosensing technologies that allow biomedical diagnostics and therapy at the molecular and cellular level. The technology involves interactions of laser radiation with metallic nanoparticles, inducing very strong enhancement of the electromagnetic field on the surface of the nanoparticles. These processes, often called ‘plasmonic enhancements’, produce the surface-enhanced Raman scattering (SERS) effect. Gold nanostars offer unique plasmon properties that efficiently transduce photon energy into heat for photothermal therapy. Nanostars, with their small core size and multiple long thin branches, exhibit intense two-photon luminescence, and high absorption cross sections that are tunable in the near infrared region with relatively low scattering effect, rendering them efficient photothermal agents in cancer therapy. Furthermore, a theranostic nanoplatform construct was created, allowing SERS imaging and photodynamic therapy. The use of the cell-penetrating peptide, TAT, greatly enhances particle uptake by the cells, allowing for the imaging and detection of a specific target, followed by localized release of therapeutic agents. In this way, theranostics can greatly improve the specificity and selectivity of various treatments, increasing efficacy while reducing unwanted side effects.

Acknowledgments. Supported in part by the Defense Advanced Research Projects Agency (HR0011-13-2-0003), the National Institutes of Health (T32 EB001040) and the Duke Exploratory Project. The content of the information does not necessarily reflect the position or the policy of the Government, and no official endorsement should be inferred.
In this presentation we discuss new approaches using fluorescence spectroscopy to understand cell behavior. The use of fluorescence to probe biological systems offers unprecedented sensitivity in vitro. Our work with fluorescence correlation spectroscopy (FCS), fluorescence anisotropy, and single molecule/particle spectroscopy has been used to understand the mechanisms of cell death and survival in a host of disease states. We discuss the study of apoptosis signaling in cell ischemia and reperfusion injury, cancer cell drug resistance, and other biological problems. We have used fluorescence imaging to understand the role of oxygen restoration in hypoxic environments. We have also elucidated the effects of oxygen on the temporal dynamics of cancer cell response to a drug. Our studies show that in ischemic tissue, apoptotic preconditioning requires the restoration of oxygen. In cancer drug studies, we have shown a marked resistance to anti-cancer compounds in the absence of oxygen. These and other studies can be used to guide therapies and drug design in the future. Coupled with microfluidics and separation techniques, a more complete picture of cell response can be elucidated. We also discuss new probe design and novel spectroscopic approaches to delving into cell function.
A major limitation of nanosensors is irreproducible and/or changing surface functionality. 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 synthesis of standard optically-active, solution-phase noble metal nanostructures and their applications using 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, these results could be expanded for different nanomaterials cores, molecular targets, and biosensor-based detection platforms.

Keywords: Biosensors, Nanotechnology, Raman, Surface Enhanced Raman
Application Code: Bioanalytical
Methodology Code: Sensors
Biomedical Samples and Sensors

Protein Expression Profiling of Signal Transduction Pathways in Cancerous Tissues Using Microring Resonator Arrays

Microring resonator arrays – an emerging technology that enables rapid, multiplexed quantification of a diverse subset of biomolecules – are used to quantify protein expression levels in cancer cell lysate and tissue homogenates for studies of aberrant signal transduction mechanisms relating to rapid proliferation and disease progression. The method seeks to improve upon current methods used to categorize cancers into distinct sub-classifications, which often rely upon genomic information, by identifying specific functional proteomic perturbations that lead to the cancer's unregulated proliferation and metastasis. Though genotypic information has increased in popularity over the past decade due to greater accessibility and decreased cost, gene mutations alone provide an incomplete picture of the tumor microenvironment. Our goal is to complement genotype-level information with protein expression levels corresponding to the oncogenes of interest. Phenotypic information of cancer specimens provides clinical value and expands basic biological underpinnings of aberrant signal transduction processes in cancerous tissues. For instance, changes in protein levels can serve as markers of disease progression and can be useful in assessing the success of a given treatment regimen. Additionally, the protein expression profile can provide further information about preferred mechanisms of signal transduction in a diseased state.

Support: NSF Graduate Research Fellowship Program and the National Institutes of Health

Keywords: Biological Samples, Biosensors, Immobilization, Protein
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
There is growing evidence that concentrations changes rather than appearance of unique disease biomarkers are important of clinical application of breath analysis. As physiological effects may cause sudden and profound changes of exhaled VOC concentrations, online and real time analytical methods have to be applied to assess these issues.

This study was intended to investigate how concentration profiles of exhaled biomarkers were affected by breath holding. Continuous breath resolved PTR-TOF-MS measurements were carried out in 20 healthy volunteers. Sampling for PTR-TOF analysis was achieved in a side stream mode with a sampling flow of 30 ml/min and a time resolution of 200 ms. After one minute of tidal breathing with a respiratory rate of 10 participants held their breath for 10, 20, 40, 60 sec and as long as possible. Afterwards the participants continued to breathe for another minute. Five selected VOCs were quantified in inspired and alveolar air by means of a custom made data processing algorithm.

Isoprene concentrations showed an increase of up to 18% when breath was hold for 10 sec and an increase up to 90% when breath was hold for 90 sec. Concentrations of Ethanol, Dimethylsulfide and Isopropanol were increased up to 49%. In contrast acetone concentrations remained constant. After 3 to 5 breaths all concentrations returned to initial values.

Effects of breath holding onto exhaled VOC concentrations depended on physico-chemical properties of the substances. Therefore, breathing patterns have to be taken into account if exhaled substances are interpreted in terms of biomarkers.
There has been a growing trend in bioanalysis toward the utilization of micro extraction techniques for sample isolation and transportation. Not only is there an interest in cost reduction associated with sampling, but also ethical advantages and improving sample retention using microsampling techniques. Major focus has been on utilizing dried blood spot (DBS) media as an inexpensive alternative to terminal blood draws. Though DBS does offer benefits towards cost reduction, shipping and sample storage, it is not without limitation specifically with respect to blood hematocrit levels. The purpose of this study is to explore the utility of solid phase micro extraction (SPME) as an alternative sampling device to DBS media cards. Bio-SPME, as described as functionalized particles bound to a core fiber substrate, enabling direct micro sampling of biological matrices without the need for additional sample treatment.

A simple set of model compounds is used to explore extraction efficiencies, detection limits, binding issues, and also hematocrit impact differences between DBS and Bio-SPME sampling techniques. Blood samples with ranging hematocrit levels are extracted using both techniques for comparison of analyte detection and overall sample cleanliness. Parameters such as extraction conditions, desorption solvent optimization and desorption times are detailed. Results demonstrate the capability of analyzing sub ng/mL concentration levels of mixed drugs in whole blood using both techniques. Whole blood samples extracted using the Bio-SPME technique exhibited increased analyte response, while demonstrating significant reduction in detected endogenous matrix as compared to DBS.

Abstract Text

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Accurate pH Measurement with pH Sensors on the Basis of an Ionic Liquid Salt Bridge

Accurate determination of pH is of fundamental importance in not only science and technology but also many facets of our life and environments. Potentiometry by use of a pH glass combination electrode equipped with a concentrated KCl salt bridge (KClSB) has been used as a reliable and convenient method of pH measurements. However, the potentiometry with KClSB is not accurate enough for solutions of very low, as well as high ionic strengths. In this work, we show that pH values of 20-200 \( \text{mol dm}^{-3} \) H\(_2\)SO\(_4\) solutions and are estimated more reliably by use of the electrochemical cell with an ionic liquid salt bridge (ILSB) that consists of tributyl(2-methoxyethyl)phosphonium bis(pentafluoroethanesulfonyl)amide (TBMOEPC2C2N) than those by use of KClSB-type. Table 1 lists the difference between the experimental and theoretical pH values in 20-200 \( \text{mol dm}^{-3} \) H\(_2\)SO\(_4\) solution obtained by use of ILSB type pH glass combination electrode. Metcalf reported that the error in the case of potentiometric pH measurements of 50 \( \text{mol dm}^{-3} \) sulfuric acid was 0.055 ± 0.05 pH (positive bias ± two standard deviations) due to the residual liquid junction potential (RLJP) at the contact of KClSB with the sample solution. The ILSB-type thus determines more accurately pH of the dilute sulfuric acid than KClSB-type. However the experimental pH values, pH\(_{\text{ex}}\), at five different concentrations are still higher than the corresponding calculated pH values, pH\(_{\text{cal}}\), by 0.005 to 0.032 pH unit.

The pH values of standard buffer solution were accurately determined by use of micro pH sensor equipped with ILSB, which fact opens the way for accurate pH determination of ultralow volume samples potentiometrically.

Keywords: Biomedical, Contamination, Electrodes, Potentiometry
Application Code: Biomedical
Methodology Code: Sensors
Biomedical Samples and Sensors

Up-Regulating Quorum Sensing Molecules for Early Detection of Bacterial Infections Electrochemically

**Abstract Text**

*Pseudomonas aeruginosa* is one of the leading causes of gram-negative bacterial infections in the hospital setting. Unique to this species is its production of pyocyanin, a redox-active quorum sensing molecule linked to biofilm formation. Because it is redox-active, pyocyanin can be detected electrochemically through the use of microfabricated nanofluidic electrode assemblies (NEAs). The sensitivity of the NEA is limited by the concentration of pyocyanin present in the sample. Recently published literature has shown that the addition of amino acids upregulates the biofilm formation of *P. aeruginosa*. However, these studies do not address the link between pyocyanin production and *P. aeruginosa* growth. The aim of this study is to electrochemically detect the up-regulation of pyocyanin production using NEAs, leading to a better understanding of how this bacterium grows at the onset of infection as well as improved detection sensitivity.

Six different amino acids and two antibiotics have been chosen as means of regulating *P. aeruginosa* cell growth. Cultures of *P. aeruginosa* are grown overnight and inoculated into separate liquid cultures, each containing a different amino acid at concentrations found in typical *P. aeruginosa* infections. Liquid samples are taken from each culture at set time intervals and loaded onto an NEA to detect the concentration of pyocyanin electrochemically using differential pulse voltammetry. The data from this study will provide a clear understanding of how pyocyanin production is linked to the up-regulation of biofilm formation in *P. aeruginosa* and result to an electrochemical technique for early infection identification. Most importantly, the concept of detecting rapidly produced small molecules by cells as a means of increasing sensitivity can be extended to virtually any pathogen.

This work was supported by the U.S. National Science Foundation under Grant #1125535.

**Keywords:** Amino Acids, Biosensors, Detection, Electrochemistry

**Application Code:** Biomedical

**Methodology Code:** Sensors
Large Scale Fabrication of Polymer Multilevel Nano-Microfluidic Lab-on-Chip (LoC) Systems for Electrochemical Sensing

Detection of currents as low as a few pA with high performances in terms of signal-to-noise ratio combined with higher spatial and temporal resolution has recently gained more and more interest. This is due to the requirements of ever decreasing detection limits in electrochemical sensing [1]. Moreover, the requests for an increased portability of sensing devices and of lower sample volumes require electrode downscaling. For this purpose, several kinds of micro and nanofabricated electrodes have been made and are described in literature. Such electrodes have been made either with standard metals or with other materials such as conducting polymers, pyrolyzed photoresist, nanowires, nanoparticles, graphene and carbon nanotubes [2, 3].

We here present a simple method for fabrication of both metal (Au) and conductive polymer microelectrodes embedded in topas™ polymer substrates. The embedding of the electrodes in the polymer substrates avoids the deterioration of the electrodes. The latter can occur due to thermal bonding in the areas where the electrodes enter the channel. The polymer substrates are then subsequently aligned and thermally bonded to injection molded microfluidic topas™ chips for LoC applications thus making the whole process attractive for large scale production. The electrodes are also characterized through electrochemical detection of ferrocene methanol in different concentrations.


Keywords: Biosensors, Electrochemistry, Electrodes, Lab-on-a-Chip/Microfluidics
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Biomedical Samples and Sensors

Optical Detection of Hepatitis Virus Proteins Using Waveguide-Mode Sensors

Viral hepatitis is one of the worldwide-spread virus infectious diseases. Among kinds of hepatitis viruses, rapid detection of hepatitis B and C viruses in human blood are especially important since those viruses should be examined on the test as a safety control before an operation. We propose the use of a waveguide-mode sensor for the rapid and sensitive detection of the hepatitis viruses. The waveguide-mode sensor is a high-speed and highly-sensitive bio-chemical sensors which utilizes a resonance of incident light in a waveguide layer of the sensor chip. The sensor chip consists of the waveguide layer on the top, a reflectance layer, and a glass substrate. Attachment of a substance at the surface of the waveguide layer yields changes in the vicinity of the surface and results in a shift of a resonant peak wavelength. The substance can be detected by observing the resonant peak shift. The viruses are detectable by using the sensor chips with appropriate antibodies immobilized at the sensing surfaces. We performed detection of hepatitis B and C virus proteins using the waveguide-mode sensor and microchannels. The proteins at a concentration of 100 nM in phosphate buffered saline are detected through the shifts of resonant peak wavelengths. Details of the measurement setup and experimental data will be presented in the paper.

This work was partly supported by SENTAN, Japan Science and Technology Agency (JST).

Keywords: Biomedical, Biosensors, Sensors

Application Code: Biomedical

Methodology Code: Sensors
MicroRNAs (miRNAs) are small (22 nucleotides) non-coding RNAs that regulate gene expression post-transcriptionally. Since deregulation of miRNAs (i.e. up or down regulation) in cancer cells are thought to be an indication for the disease, detection of miRNAs is very important for early diagnosis and treatment of most of the cancer types. Due to low abundance inside the cell and high similarity between other miRNAs, detection of specific miRNAs is challenging. Enzymatic detection of mir 21 has been done[1]. In this study, a novel electrochemical biosensor was designed for the detection of mir21 from cell lysates based on the oxidation signal of tryptophan amino acid inside virus encoded, RNA silencing protein ; p19. Due to size specific recognition property of p19, target miRNA is detected in a direct, label-free, rapid and inexpensive way upon hybridization with its complementary target followed by p19 interaction[2]. The altered tryptophan oxidation signals after and before the interaction with hybrid miRNA, is the indication of the detection and the conformational change of p19.


Keywords: Biosensors
Application Code: Biomedical
Methodology Code: Electrochemistry
Serious health complications and fatal overdoses have brought phenethylamine designer drug use to the public’s attention. The purpose of this work is to determine not only the identity of the psychoactive compound/s, and their concentrations in the various street samples, but also to determine impurities which may exist from less than ideal synthetic procedures. These impurities are thought to be the cause of serious health complications. These synthetic drugs are marketed as having affects similar to LSD and MDMA and are typically consumed sublingually via blotter paper. The compounds have a variety of street names including “N-BOMB,” “Smiles,” and “Bromo-DragonFLY.”

The objective of this research is to qualitatively and quantitatively identify the drugs and potential impurities. Street samples will be compared to known standards in order to determine if impurities exist that may be resulting in health complications. The analysis will be done using a variety of ultra-high performance liquid chromatography (UHPLC) instruments. Following UHPLC separation, time of flight mass spectrometry will be employed for compound identification. Additionally, a photo diode array detector will be coupled, in-line, to aid in quantification. Finally, a separate sample introduction technique (direct sample analysis) will be coupled thus providing various methods of analysis and identification of the targeted drugs and impurities which will all be compared and contrasted in this presentation.

MS/MS spectra were used to determine the fragmentation patterns. While the MS/MS spectra for the various compounds were similar in fragmentation patterns, they also displayed differences that allow the analyst to distinguish which compound is present. These fragmentation patterns, in addition to accurate mass TOFMS, will be used to qualitatively identify street samples and also determine impurities.

**Keywords:** Drugs, Forensic Chemistry, Liquid Chromatography/Mass Spectroscopy

**Application Code:** Drug Discovery

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Discovery of small molecules that bind to protein targets is an essential step in designing novel therapeutic agents. Label-free biosensor technologies such as surface placement resonance spectroscopy have become routinely used techniques to discover and quantify protein-legend interactions. Here we demonstrate the utility of manpower optical interferometer (NPOI) biosensor interfaced to mass spectrometry for small molecule drug screening, protein-protein interactions and the ability to simultaneously generate binding kinetics, affinity parameters, MS and MS/MS-based identification.

A target of interest is immobilized to the NPOI 3D-surface (average pore size 80 nm; effective area of 400,000 nm^2) for example carbonic anhydrase and mixtures of ligands (furosemide, sulpiride, naphthalenesulphonemide, sulphanilamide) flow across the surface; the eluent from the biosensor surface is then directly injected onto a reversed phase column for accurate mass LC-MS(MS) analysis. Protein-protein interaction analysis is performed on the same platform. A two-valve switching system has also been constructed allowing the sample and reference eluents to be collected and independently sampled by LC-MS(MS).

Using the carbonic anhydrase-furosemide system, legend dilution ranges are 20 µM to 150 nM and a dissociation constant (KD) of 1.88 µM is derived, comparable to the SPR derived value of 0.516 µM. Accurate MS and MS/MS can also be performed simultaneous to the enzyme kinetic measurements. The total turnaround time for one binding-dissociation-MS experiment is 15 mins. We also describe the concomitant acquisition of protein-protein kinetic interaction information and intact protein MS-detection and quantitation.

This platform has the potential to be utilized for many "fishing" types of experiments.

Keywords: Biotechnology, Drug Discovery, Liquid Chromatography/Mass Spectroscopy
Application Code: Drug Discovery
Methodology Code: Microfluidics/Lab-on-a-Chip
Drug Discovery

Accelerating Drug Discovery Using Capillary Electrophoresis as a Pre-Screening Tool for High-Throughput Analysis

The molecular discovery technology described herein couples capillary electrophoresis (CE) pre-screening with a final “lab-on-bead” selection method. Specifically, this work seeks to select ligands in small molecule libraries against clinically relevant targets such as tyrosine kinase proteins. Ultimately, DNA-encoded small molecule libraries containing many thousands of potential drug candidates will be mixed with protein targets of interest, and a modified CE method will be used as a preselection tool to isolate only those ligands that most strongly bind the target. An efficient separation method has been developed that utilizes capillary transient isotachophoresis (ctITP) for the separation of bound from unbound ligands using a model thrombin protein (target) and 29-base thrombin aptamer (ligand) system. Whereas CE alone resulted in broad peaks with low sensitivity for the protein-aptamer complex and free aptamer, the new ctITP method with laser-induced fluorescence (LIF) detection developed in this work, which incorporated high fidelity, on-column labeling of the aptamer by the SYBR Gold fluorescent probe during the course of the separation, yielded enhanced signal and resolution, suitable for the automated collection of bound ligands to feed into subsequent chip-based assays. Collected fractions of the bound 29-mer thrombin aptamer obtained from a spiked, randomized ssDNA sample were sequenced using the automated Ion Torrent[circumflex O] personal genome machine. In this way, the aptamer identity and a 100-fold sample enrichment were validated for the CE-based prescreening or molecular selection process. Application of these coupled elements to other ligand selections for drug discovery will follow. This work is supported by NCBC Grant #2011-MRG-1115 and Wake Forest University.

Abstract Text

Keywords: Capillary Electrophoresis, Drug Discovery, Fluorescence, High Throughput Chemical Analysis

Application Code: Drug Discovery

Methodology Code: Capillary Electrophoresis
Law enforcement already integrates the use of GC-FID to test for cannabinoid potency in marijuana samples. Given the legalization of medical marijuana, a need to better analyze marijuana samples is highly desired. While potency is monitored to determine strength of medical samples, it is thought that fingerprinting pesticides present in each sample could be highly important. Experience has shown that most cannabis is treated with various pesticides, fungicides, and insecticides depending on the grower. The presence of these organic pesticides could present a safety issue, as they would not be ideal to have in medical marijuana samples. Additionally, the use of certain pesticides varies widely between samples, so pinpointing exactly which pesticides are in each sample could help identify a specific source for the sample and aid in the determination of trafficking patterns.

Cannabinoids and organic pesticides appear in marijuana sample chromatograms at very different ratios/concentrations. Given the large size of the cannabinoid peaks compared to the pesticides, it can be hard to simultaneously measure the pesticides with a standard chromatographic analysis. It is thought that using a Deans Switch would help “cut” out the larger cannabinoid peaks so that a GCxGC pesticide fingerprint could be determined for each sample, simultaneous to the cannabinoid profile.

This method integrates a Deans Switch into the GCxGC-FID/ECD, which allow for the GC-FID analysis of the cannabinoids for potency while the GCxGC-ECD provides the pesticide fingerprint in one method. Also, investigations into using a TOFMS system, in addition to the FID/ECD approach will be discussed to compare and contrast the two approaches. Optimizing one method that could determine potency and fingerprint trace pesticides could be instrumental for law enforcement to determine sources for various street and medical marijuana samples with a single method that may reduce costs and time.

**Abstract Text**

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**Keywords:**
Drugs, Gas Chromatography, Pesticides

**Application Code:**
Drug Discovery

**Methodology Code:**
Other (Specify)
Rapid identification of sites of metabolism of prospective drug candidates expedites rational chemical structure design in discovery programs. Speed and sensitivity make LC/MS/MS a core technology for such identifications, with efficiently targeted approaches gaining appeal to obviate the data burden. So software capable of predicting metabolite structures from relevant assays and then mass spectrometry data processing and interpretation can occur in at least a semi-automated manner is needed.\[1\] We describe new software, predicting structures for human metabolites of CYP450 enzymes and then enabling their targeted component detection by analysis of LC/MS data. Additional tasks of structure and results databasing and reporting is also simplified.

A tree of phase I metabolites of a particular compound is obtained utilizing the information about which of its atoms are the most likely targets for either of the five most popular isoforms of human cytochrome P450 (CYP3A4, CYP2D6, CYP2C9, CYP2C19, CYP1A2). The probabilities of being a site of the biotransformation are provided by a variant of fragmental pharmacophore-like models.\[2\] Novel GALAS modeling methodology made possible a quantitative reliability estimation for each prediction. A commercial analytical software platform was modified slightly, providing in its Table of Components of the processing interface a user-selected precursor molecule plus metabolite structures and formula, with corresponding masses and m/z values used for targeted chromatogram extraction. Components matching detected peaks had retention times automatically assigned, after further refinement for situations where isomers and/or multiple peaks were indicated. Where structural ambiguity remained, a novel feature enabled automatic collapse into a single Markush representation for better depiction.

\[1\] H T'jollyn et al. Drug Metab Dispos. 2011, 39, 2066.

**Keywords:** Automation, Data Analysis, Drug Discovery, Software

**Application Code:** Drug Discovery

**Methodology Code:** Data Analysis and Manipulation
Drug Discovery

Natural Hydrogel/membrane Structures and Lipogels as Drug Delivery Systems

A lipid bilayer membrane supported by elastic polymer network (hydrogel) is the one of unique achievements of Nature in constructing multifunctional, flexible, and dynamic machineries, called cells. Diversity of natural hydrogel/lipid membrane structures in bacterial cell envelopes is shown and their types are systemized as prototypes of a new class of drug delivery systems. Artificial spherical configurations of the lipid bilayer/hydrogel assembly, called lipogels, were synthesized by two ways: polymerization within liposomal reactor and nanogel-liposome mixing. New properties of thus prepared lipogels were discovered. Bipartite structure of lipogels makes them attractive as stable drug delivery containers with capability of controlling load release. In particular, it was shown that reversible and irreversible aggregation of lipogels can be used for designing two types of combined multifunctional containers: (i) different drugs entrapped in different lipogels can be simultaneously delivered as one aggregate to the targeted organs in the body and released in desired order; (ii) several nanogels loaded with different pre-drug reagents are trapped under the one lipid membrane (“giant lipogel”) to react inside without damaging surrounding organs and to be delivered to the targeted site in this “giant” container. Novelty and advantages of the drug delivery systems, which use the hydrogel/lipid membrane assemblies as structural elements borrowed from bacterial cell envelopes, are discussed. KEYWORDS: bacterial cell envelope, hydrogel/lipid bilayer assembly, lipogels, drug delivery systems.

Keywords: Biomedical, Membrane, Nanotechnology, Polymers & Plastics
Application Code: Nanotechnology
Methodology Code: Microscopy
Nitric oxide (NO) plays an important role in regulating vasodilation in the brain. Carbon monoxide (CO) is also a physiologically important signaling molecule, which has analogous functions to NO. The exact functional relationship between NO and CO, however, has not been explained clearly yet, due to technical difficulties of measuring NO and CO simultaneously. To better understand the dynamic and close interactions of NO and CO, we have developed simultaneous real-time NO and CO measurements by using electrochemical dual microsensor [1]. In this study, we further improve the amperometric dual microsensor and optimize simultaneous monitoring of dynamic changes in NO and CO levels in vivo.

The sensor consists of a planar working electrode possessing two Pt microdisks (diameter of each disk $50 \, \mu m$) electrodeposited with second metals (Pt black, Sn, Pt/Fe) and a Ag/AgCl reference electrode. Each disk surface of the working electrode is covered with gas permeable polymer membrane to obtain the selectivity to only NO or CO over common biological interfering species. The small size and facilitated gas transport through the sensor membrane allow to measure NO and CO with high spatiotemporal resolution and thus improve our NO/CO dual sensor performances in vivo. The NO/CO dual sensor is applied to monitor dynamic changes of NO and CO levels in normal as well as dysfunctional rat brain. Our particular interest is to assess the effect of neuronal excitation in the interaction between NO and CO in seizure model.

This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Ministry of Education, Science and Technology (NRF-2011-0015619).


Keywords: Bioanalytical, Biosensors, Electrochemistry, Microelectrode
Application Code: Bioanalytical
Methodology Code: Sensors
The measurement of glucose in surrogate fluids has long been proposed as a non-invasive supplement to diabetic blood sampling [1]. Our group has previously reported on correlations between tear and blood glucose concentrations in anesthetized rabbits using needle-type electrodes with sample tear volumes of 3 [micro]L [2]. Conventional blood glucometers utilize amperometry for glucose quantification, but detection ranges for these devices lie well above fasting tear glucose concentrations (20-200 [micro]M) [3]. We have recently found that some commercial blood glucometer strips requiring [less than] 1 [micro]L of sample can detect tear glucose in these low quantities using a high current sensitivity laboratory potentiostat (BioStat, ESA Biosciences). In this presentation, a new handheld, ultra-sensitive, and low-cost glucometer will be described that is capable of measuring tear glucose using commercially available blood glucose strips. By employing a buffered feedback ammeter, a theoretical resolution of 0.3 nA and dynamic range of 0-333 nA was obtained. After calibration of the glucometer with fresh solutions of glucose in PBS, bench-top verification was conducted with samples containing known electroactive interferents. Preliminary human tear testing data showed agreement between values obtained from the tear glucometer and laboratory potentiostat. The proposed tear glucometer will help facilitate future large-scale clinical studies to assess the effectiveness of tear glucose testing as a supplement to regular blood glucose monitoring in diabetic patients.

1.) Khalil OS. Diabetes Technology & Therapeutics. 6(5), 2004.

**Keywords:** Bioanalytical, Electrochemistry, Instrumentation, Portable Instruments

**Application Code:** Bioanalytical

**Methodology Code:** Portable Instruments
The brain is composed of a complex vascular system that directs nutrient-rich blood to areas of increased activity. Studies have demonstrated that neurotransmission triggers these blood flow changes by influencing local vascular tone. While the failure of this process to match local energy needs underlies a number of disease pathologies, much remains to be learned regarding the identity and signaling pathways of the chemical messengers involved. Here we consider the vasoactivity of the catecholamine norepinephrine within the brain. In the periphery norepinephrine acts to constrict blood vessels, and it is believed that it may retain this function in the central nervous system. The presence of norepinephrine terminals near cerebral arterioles and capillaries further supports this theory.

Using oxygen as indirect measure of blood flow, we employed fast-scan cyclic voltammetry (FSCV) at carbon fiber microelectrodes to investigate the noradrenergic mechanisms of cerebral hemodynamics in the ventral bed nucleus of the stria terminalis (BNST). The waveform used had an anodic limit of 0.8 V and a cathodic limit of -1.4 V, allowing simultaneous detection of norepinephrine and oxygen via their distinct redox processes. We found that norepinephrine release by electrical stimulation or iontophoretic ejection triggered a rise in oxygen levels followed by a brief dip below baseline. Iontophoresis of selective norepinephrine drugs demonstrated that each component of this biphasic response was due to local receptor mechanisms. Overall these results support that norepinephrine release in a deep brain region can mediate both the onset and termination of a hyperemic event.

Keywords: Bioanalytical, Biosensors, Neurochemistry, Voltammetry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Reference electrodes are an inevitable part of almost any electrochemical measurement. In many reference electrodes, nanoporous glass plugs are used to contain the electrolyte solution that forms a salt bridge between the sample and the reference electrode. Although reference electrodes with nanoporous glass plugs (such as Vycor or CoralPor glass) are commercially available and are frequently used, the limits of their use have not been investigated thoroughly. It is shown here that at low ionic strength, the half-cell potentials of reference electrodes with liquid–liquid junctions to electrolyte solutions supported by nanoporous glass are not sample independent and depend on the sample composition, both in aqueous and organic solutions. Reference potentials were found to be affected by more than 50 mV in aqueous solutions by HCl, NaCl, KCl, and CaCl$_2$. Also, in acetonitrile, a representative organic solvent, reference potentials were affected by CF$_3$COOH and the supporting electrolyte tetrabutylammonium perchlorate.

It is shown that these variations in the reference potential cannot be explained by the liquid junction potential between two mutually miscible electrolyte solutions, as described with the Henderson equation. Instead, 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. Novel inorganic and organic plug materials are suggested to be used in reference electrodes to avoid this problem. When using reference electrodes with nanoporous plugs, their limitations should be considered to avoid substantial errors in the measurement, e.g., by selecting electrolyte conditions carefully.

# Electrochemical Sensors for Bioanalysis

## Biocompatibility Strategies for Intravenous Continuous Glucose Monitoring Sensors

Blood glucose in diabetic patients can exceed the 4.4 - 6.6 mM range seen in healthy individuals and is commonly measured in medical centers with point-of-care instruments, or at home with finger prick glucometers. While these devices yield discrete blood glucose values, rapidly increasing/decreasing glucose concentration trends are often unobserved. Several commercial devices provide continuous glucose monitoring of interstitial fluid utilizing implanted electrochemical sensors. However, in practice, these only supplement, rather than replace discrete blood measurements. Intravenous amperometric glucose sensors may provide better platforms for continuous blood glucose measurements, especially in a hospital environment. Indeed, tight glycemic control is a requirement in many intensive care units (ICUs) to achieve better patient outcomes. There are several challenges to achieve continuous and accurate intravenous blood glucose measurements. Other electroactive species present in blood can also be oxidized at the sensor’s surface, causing errors. Furthermore, use of outer amphiphobic polymeric layers limits glucose diffusion to the sensor surface to achieve linearity over the wide range of blood glucose concentrations associated with diabetic patients. The central problem facing intravascular sensors is the tendency for clot formation on their surfaces. Nitric oxide (NO) release by diazeniumdiolates or other species incorporated within the outer coating can prevent thrombus formation, and improve the sensor’s functional lifetime. Further, immobilization of a recombinant transmembrane protein known as CD47, can also reduce platelet activation. It will be shown that these sensor design improvements allow intravenous amperometric sensors to combat physiological responses that normally limit their [i]in vivo[/i] analytical performance.

## Keywords
- Bioanalytical
- Biosensors
- Electrochemistry

## Application Code
- Bioanalytical

## Methodology Code
- Electrochemistry
Oxygen (O$_2$), nitric oxide (NO) and carbon monoxide (CO) are all important biological active gases in regulating fundamental physiological function. Endogenous CO and NO have similar biological functions controlling vasodilation and neuronal function although high concentrations of CO and NO have biotoxicity. Besides, the biosynthetic procedure of NO and CO is also alike in the way that both synthetic enzymes need O$_2$ and NADPH to be activated. Furthermore, whenever the neural system encounters transient hypoxia, NO and CO are endogenously generated to meet the increased metabolic demands, i.e. O$_2$ demands. In this study, for the first time, we demonstrate the development and biological application of an amperometric O$_2$/NO/CO triple microsensor which can measure these gases simultaneously in real-time in vivo. The sensor consists of a triple planar working electrode (WE) and a Ag/AgCl counter/reference electrode. The triple disk WE possessing three platinum microdisks (WE1, WE2 and WE3, 76, 50, 25 μm in diameter, respectively) is etched to form micropore-shaped electrode and then electrodeposited with porous platinum layer. The largest WE1 is additionally modified with electrochemical deposition of tin. Modifications of three sensing disks different in their sizes as well as in their disk surface treatments result in dissimilar selectivity to each gas at WE1, WE2, and WE3, enabling the gas differentiation one from the others. For the sensor feasibility test, the prepared sensor is applied for real-time simultaneous measurements of the dynamic changes of O$_2$/NO/CO levels in living mouse organs (e.g., brain, kidney). This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Ministry of Education, Science and Technology (NRF-2011-0015619).
Electrochemical Sensors for Bioanalysis

Quantitative Detection of Fucoidan Using Polyion-Sensitive Electrochemical Sensors

Fucoidans are a class of sulfated polysaccharides derived primarily from brown seaweeds, exhibiting significant biological activity and potential therapeutic use. Fucoidans have been shown to decrease osteoarthritic pain, inhibit retroviruses, prevent tumor cell growth, modulate blood coagulation and protect against renal and kidney disease.[sup]1[/sup] This presentation describes the use of electrochemical polyion sensors operating under non-equilibrium, steady-state conditions for the quantitative measurement of fucoidan from several different species of macroalgae, including Fucus vesiculosus and Undaria pinnatifida. Although polyion sensors have previously been used for detecting linear polyions, this research demonstrates one of the first applications of polyion sensors for quantitative measurement of highly branched macromolecules. The response characteristics of polyanion sensitive electrodes incorporating tridodecylmethylammonium chloride (TDMAC) ion-exchanger will be presented. It will be shown that sensor response varies for different species of fucoidan, and can be used to characterize fucoidan samples according to their charge density, peak molecular weight and degree of branching. Fucoidan can also be measured via potentiometric titration with protamine, monitored with a polycation sensitive electrode incorporating dinonylnaphthalene sulfonate (DNNS) ion-exchanger. The binding ratios between protamine and different species of fucoidan have been determined, yielding further insights regarding structural differences between the fucoidan extracts. Polycation sensitive electrodes have been used to accurately measure the fucoidan content of several commercially available nutritional supplements via potentiometric titration. Future work may expand the use of these sensors for monitoring the activity of fucoidanase enzymes and measuring therapeutic doses of fucoidan in biological fluids.


Keywords: Bioanalytical, Detection, Ion Selective Electrodes, Sensors
Application Code: Bioanalytical
Methodology Code: Sensors
A field-deployable electrochemical approach to preconcentration, matrix clean up and electrodeposition of Pb in potable water will be described. Using portable, battery-operated potentiostat, Pb was electrodeposited in the field on portable coiled-filament assemblies made from Re. The assemblies with dried Pb residues on them were transported to the lab for concentration determination by electrothermal, near-torch vaporization (NTV) sample introduction and inductively coupled plasma atomic emission spectrometry (ICP-AES). This is different from the Cr(III)/Cr(VI) speciation we presented last year at PittCon 2013.

Keywords: Atomic Emission Spectroscopy, Environmental Analysis, Plasma Emission (ICP/MIP/DCP/etc.)
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Environmental Analysis of Metals in Water

Simultaneous Atomic Absorption and Atomic Fluorescence Spectrophotometry for Mercury Determination in Water Samples

There are more than 1300 commercial testing labs in the US with one out of three samples tested related to water. With EPA regulated methods such as 1631, 245.7 and 245.1, emphasis on mercury levels and analysis has never been stronger. These methods suggest mercury analysis on two different instruments, Cold Vapor Atomic Absorption Spectrophotometry (CVAAS) and Cold Vapor Atomic Fluorescence Spectrophotometry (CVAFS). Most labs in compliance with these regulations analyze their samples on the two separate systems mentioned, consequently reducing their productivity, increasing waste and reagent costs and maintenance of two systems. This presentation will illustrate a novel way of analyzing mercury in water samples, simultaneously incorporating both CVAAS and CVAFS in a single system.

The presentation will also provide insight into:

*Several challenges analyzing mercury in water samples
*Technical discussion around simultaneous CVAAS and CVAFS measurement
*Principle of operation and key features in FMA-80/AAS system
*Experimental Hg data analysis
*Increasing productivity and ROI for water testing labs

Keywords: Atomic Spectroscopy, Environmental/Water, Fluorescence, Mercury
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Mercury is among the most toxic elements and the toxicity, bioavailability, and transportation depends on the mercury species. Organic mercury is known as the most toxic species and has a higher bioavailability compared to divalent mercury. Life on earth is based on water and mercury in water ends therefore up in biota. The analysis of water samples for mercury is therefore important but the concentrations of mercury species in natural water are lower than the sensitivity of the analytical methods. The mercury species are therefore pre-concentrated on a pre-column and then eluted using liquid chromatography coupled to UV photolysis and cold vapour fluorescence spectrometry. The method was successfully applied for biological samples (e.g. hair, fish). The advantage of the method is a minimum of sample preparation prior to analysis. The optimisation study and analytical performance will be reported.

Keywords: Environmental/Water, Mercury, Speciation, Water

Methodology Code: Atomic Spectroscopy/Elemental Analysis
A novel approach to generating a localised \textit{in situ} pH change is investigated, with the aim of improving heavy metal electroanalysis. Electrochemical deposition and stripping of heavy metals is aided by low pH conditions, however measurement \textit{in situ} e.g. in rivers or lakes, often takes place away from ideal conditions. In this study, the ring electrode of a boron doped diamond (BDD) ring disc electrode was used to generate protons via the electrochemical decomposition of water. This results in the controlled adjustment of local pH to the disc electrode where metal deposition and stripping detection occurs. The system was used to investigate mercury deposition/stripping, using cyclic voltammetry, in pH neutral solutions. The local pH change, as a function of galvanostatically applied current was characterised using an iridium oxide film on the BDD disc electrode. Our results showed that it was possible to generate an electroanalytical stripping signature for mercury in a pH 7 solution, similar to that in a pH 2 solution, using \textit{in situ} proton generation. This procedure thus provides a simple \textit{in situ} heavy metal analysis method, which due to the use of BDD is stable and corrosion resistant in a wide range of environments and avoids the need to remove samples from the source to acidify for further analysis.

\textbf{Keywords:} Electrochemistry, Environmental/Water, Mercury, Stripping Analysis

\textbf{Application Code:} Environmental

\textbf{Methodology Code:} Electrochemistry
Environmental Analysis of Metals in Water

Development of Highly Stable Solid Phase Reagent Strips for the Detection of Magnesium Hardness

Water hardness measurement relates to quality of the water sample. In general, industrial and residential water systems draw water from a number of potential sources such as wells, rivers and reservoirs. These sources have varied levels of water hardness. Calcium and Magnesium species are responsible for the hardness and expressed as calcium carbonate in milligrams per liter or ppm. These species also account for inorganic scaling and fouling of water samples. In residential systems, the water fouling is observed in sinks, tubs, dishes, glassware and hot water heaters and in industrial systems the water fouling is observed in boilers, heat exchangers and steam generating plants. For these reasons, hardness is an important control test.

Many companies have developed titration kits for the detection of total and calcium hardness. Colorimetric and fluorescent methods are also available for the measurement of total and Calcium hardness. However, very few methods are available for the selective Magnesium detection. The most common way of finding the Magnesium concentration is by subtracting the Calcium value from Total Hardness. The detection of Magnesium Hardness in various coolants and water samples is important to control the quality of the samples.

In view of this, we have developed a novel solid phase reagent strips for the detection of Magnesium hardness. The strip demonstrates high precision and accuracy, and a long shelf life. The developed strips are used for real time analysis in our photometers. No separate sample cell is required. Using the developed solid phase strips, we can measure the water hardness in various samples from the range 5 to 600 ppm of Mg as CaCO₃. The chemistry of the reaction, performance and stability of the test strips and the results of the various samples are discussed.

Keywords: Environmental Analysis, Portable Instruments, Sensors, Spectrophotometry
Application Code: Environmental
Methodology Code: Sensors
Manganese (Mn) has historically been regarded as a nuisance contaminant, aesthetically degrading water quality and the main cause of discoloration in drinking water. It was found that addition of ferric chloride (used as a coagulant) and H₂SO₄ (used to adjust pH) during the water treatment process gave rise to higher concentrations of manganese in water samples compared to raw waters. Once manganese entered the network it could potentially be precipitated, due to physicochemical and biological processes, and hence give rise to discoloration events. A method is described here for manganese speciation at sub-mg L⁻¹ level in drinking water samples using the hyphenated technique HPLC ICP-MS (X series 1, Thermo Scientific) with a helium (He) gas collision cell. Helium gas was useful to eliminate polyatomic interferences. Drinking water samples were subjected to pre-treatment with mobile phase (1mM Tetrabutylammonium Hydroxide + 0.6 mM EDTA (potassium salt), pH 6.9 and 2% MeOH) prior to analysis. Two reverse phase HPLC columns were tested for speed of analysis; an Agilent Zorbax Eclipse XDB-C8 and a Thermo Scientific Accucore RP-MS. Results demonstrated the presence of higher concentrations of soluble Mn²⁺ in post-treatment water samples at the treatment works, indicating that a change in water treatment may reduce soluble manganese entering the water distribution network. In terms of retention times, the Agilent column retained Mn²⁺ for 8.33 minutes whilst the Accucore RP-MS column retained Mn²⁺ for only 4.16 minutes thereby reducing argon demand and time.

Keywords: Chromatography, Environmental Analysis, Environmental/Water, Mass Spectrometry
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
Environmental Analysis of Metals in Water

Real-Time Electrochemical Detection of Arsenic

Over the last few years, Arsenic (As) pollution has been a severe environmental problem that has gained momentum due to increased exposure of the public to arsenic through water and food. Arsenic is present naturally in the environment and is further released through anthropogenic processes including pesticides, preservatives, paints and plating. Detection of As in situ is essential to mitigate its impact, however, there are a lack of green analytical methods that can be used in situ with high sensitivity and selectivity. In this paper, we describe an electrochemical method to detect As using fast scan cyclic voltammetry (FSCV). Our method is green, sensitive and performs real-time As analysis. We describe the optimization of our method and its application to real environmental systems. The ultimate objective of this project is to understand As transport from natural and anthropogenic sources into our food and water supplies so that its effects can be more efficiently mitigated.

Keywords: Detection, Electrochemistry, Environmental, Microelectrode

Application Code: Environmental

Methodology Code: Electrochemistry
Pollution by trace metals such as Cu, Cr, As, Hg and Pb is a major concern to the environment and to public health. To more effectively mitigate the impact of trace metals, there is great interest in developing a portable device that can report trace metals levels instantaneously and continuously with high sensitivity selectively. We have previously described real-time, sensitive Cu[1] and Pb[2] detection with Fast-Scan Cyclic Voltammetry (FSCV) at carbon fiber microelectrodes (CFMs). In this work, we take a novel approach to improve the selectivity of our method. By covalently modifying the carbon fiber microelectrode surface with highly selective ionophores, we marry ion-selective methods to voltammetry. This affords an extremely high level of selectivity between metals. We optimize electrode modification parameters and assess the modified electrodes’ sensitivity, selectivity and stability. Our novel modification makes fast metal voltammetry extremely powerful as a portable, selective and sensitive tool for continuous metal detection.


Keywords: Electrochemistry, Electrodes, Metals, Voltammetry
Application Code: Environmental
Methodology Code: Electrochemistry
Nanotechnology: Spectroscopy, Microscopy, and Imaging

Study of Charge-Dependent Efflux Function of Multidrug Membrane Transporters in Single Live Cells

Currently, molecular mechanisms of multidrug ABC (ATP-binding cassette) membrane transporters remain obscure. In this study, we have functionalized the surfaces of silver nanoparticles (Ag NPs, 10.7 ± 0.9 nm in diameter), with two peptides (CALNN[b]K[b] and CALNN[b]E[b]) to prepare positively (Ag-CALNNK NPs[sup]+) and negatively charged NPs (Ag-CALNNE NPs[sup]-), which were stable (non-aggregation) in PBS buffer and inside single live cells. The surfaces of single Ag NPs can be functionalized with different peptides, offering the feasibility of using them to mimic various charges of antibiotics (drugs) for probing the efflux kinetics of membrane transporters of single live cells for better understanding of multidrug resistance. We used the single Ag-peptide NPs to probe the charge-dependent transport kinetics of the ABC (BmrA, BmrA-EGFP) transporters in single live cells ([i]Bacillus subtilis[/i]) using dark-field optical microscopy and spectroscopy (DFOMS). The results show that the accumulation and efflux kinetics of intracellular Ag-peptide NPs for single live cells depended upon the charge of the Ag NPs, the cellular expression level of BmrA and the NP concentrations. In this study, we have demonstrated that single plasmonic Ag-peptide NP optical probes can serve as excellent optical probes for real-time study of efflux kinetics of multidrug membrane transporters in single live cells and for better understanding multidrug resistance.

Abstract Text

Currently, molecular mechanisms of multidrug ABC (ATP-binding cassette) membrane transporters remain obscure. In this study, we have functionalized the surfaces of silver nanoparticles (Ag NPs, 10.7 ± 0.9 nm in diameter), with two peptides (CALNN[b]K[b] and CALNN[b]E[b]) to prepare positively (Ag-CALNNK NPs[sup]+) and negatively charged NPs (Ag-CALNNE NPs[sup]-), which were stable (non-aggregation) in PBS buffer and inside single live cells. The surfaces of single Ag NPs can be functionalized with different peptides, offering the feasibility of using them to mimic various charges of antibiotics (drugs) for probing the efflux kinetics of membrane transporters of single live cells for better understanding of multidrug resistance. We used the single Ag-peptide NPs to probe the charge-dependent transport kinetics of the ABC (BmrA, BmrA-EGFP) transporters in single live cells ([i]Bacillus subtilis[/i]) using dark-field optical microscopy and spectroscopy (DFOMS). The results show that the accumulation and efflux kinetics of intracellular Ag-peptide NPs for single live cells depended upon the charge of the Ag NPs, the cellular expression level of BmrA and the NP concentrations. In this study, we have demonstrated that single plasmonic Ag-peptide NP optical probes can serve as excellent optical probes for real-time study of efflux kinetics of multidrug membrane transporters in single live cells and for better understanding multidrug resistance.

Keywords: Bioanalytical, Biomedical, Imaging, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Molecular Spectroscopy
Bioderived nanomaterials represent new approaches to generate functional structures on the nanoscale using sustainable approaches. These materials have demonstrated unique properties, especially in the areas of biosensing, catalysis, and optics; however, the structure of the biomolecules on the particle surface remains unclear where such components could have dramatic implications on the final activity of the particles. We have employed a variety of characterization methods to probe this region, where our results have indicated that localized binding effects at the amino acid level are critically important in controlling particle functionality. For this, we have employed Pd nanoparticles, capped on the surface with the Pd-specific Pd4 peptide (TSNAVHPTLRHL). Using this sequence as a surface passivant, ~2 nm Pd particles can be prepared. These materials are highly active for C-C coupling reactions and operate under atypical conditions of a water-based solvent at room temperature. High-resolution analysis of the nanoparticles using techniques such as EXAFS, QCM, TEM, CD, and UV-vis has indicated that the peptide surface structure plays an important role in controlling this reactivity. Such studies are supported by computational modeling analyses of peptide surface binding. Finally, through selective residue modification of the peptide sequence, the functionality of the materials can be substantially enhanced, thus indicating that localized surface binding effects are critically important for controlling the final activity of the nanoparticles.

Keywords: Atomic Spectroscopy, Characterization, Metals, Peptides
Application Code: Nanotechnology
Methodology Code: Chemical Methods
Design of ultra-sensitive sensors utilizing the unique optical properties of metallic nanostructures is currently an active research area. The size, shape, surface ligand chemistry, surrounding dielectric environment of the nanostructures and the electric permittivity of the supporting substrate highly influence the sensitivity of the sensors. Herein we designed the sensors based upon self-assembled monolayer of different para-substituted thiophenols on the surface of gold nanoprisms by utilizing their unique localized surface plasmon resonance (LSPR) optical properties. For the first time, we have shown that the LSPR dipole peak of gold nanoprisms was strongly tuned by modifying their surface with different para-substituted thiophenols. The charge transfer efficiency between nanoprisms and adsorbents dramatically influenced the LSPR peak shifts. The electron donating and electron withdrawing capabilities of the functional group present in the adsorbents control the charge transfer efficiency. The para-substituted thiophenolate coated gold nanoprisms can be further utilized to monitor the kinetics of various coupling reactions such as Heck coupling through monitoring the LSPR properties of nanoprisms.

**Keywords:** Materials Science, Nanotechnology, Sensors, UV-VIS Absorbance/Luminescence

**Application Code:** Nanotechnology

**Methodology Code:** UV/VIS
Superhydrophobic (or water-repellant) surfaces are used in a wide range of industrial and research applications. In several fields, surfaces are needed, which do not lose their water repellant properties after long-time immersion in water or other liquids. We here present a novel superhydrophobic surface with extremely high underwater stability made of nanometer-thick wall structures. The structures were fabricated in silicon by using a combination of deep UV Lithography, dry etching techniques and surface coating. The effect of surface pattern and roll-off direction on water drop roll-off angles and receding and advancing water contact angles was studied. The most ordered surface consisted of a lattice with square-shaped cavities separated by ~400 nanometer thick walls. By moving the lattice points randomly, increasingly irregular cavity structures were realized (see attached image). Interestingly, the lowest roll-off angle was observed for the most regular structures. On the other hand, there was a large degree of direction dependence on these surfaces. Based on our findings, the combination of underwater stability and water repellency at nano-patterned surfaces will be discussed and strategies for optimizing surface nanostructures with regard to these properties will be presented.

This study was supported by the Danish National Advanced Technology Foundation through the Large Area Nanostructuring Initiative (LANI).
The discharge of pollutants such as pharmaceuticals, personal care products, heavy metals, and other toxicants into freshwater streams and oceans is a growing environmental problem. Nanoparticles, a new class of environmental pollutants, have attracted much attention in recent years because of our ability to readily synthesize particles of controlled size, shape, and surface chemistry. The control over these properties has enabled new applications in electronic, biomedical, pharmaceutical, cosmetic, clothing, and environmental material applications. Despite their increasing industrial use, our knowledge of the environmental consequences of released nanoparticles in air, soil, and water, is wanting. Gold nanoparticles, synthesized via the seed mediated growth mechanism, have been systematically exposed to wood frog tadpoles from hatching until metamorphosis. The total uptake of gold into the wood frogs has been determined with ICP-OES and the localization of accumulated gold nanoparticles has been investigated with TEM. In addition to the chemical analysis and microscopy of the particle fate in the wood frogs, time to metamorphosis, and weight at metamorphosis have been measured.

Keywords: Environmental/Biological Samples, ICP, Microscopy, Nanotechnology
Application Code: Nanotechnology
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Stimulated emission depletion (STED) microscopy provides sub-diffraction limited imaging opportunity for fluorescent samples in situ. This opens up new opportunity in the understanding of several phenomena hitherto impossible from conventional techniques. In STED microscopy, donut shaped depletion beam is spatially overlapped with an excitation beam. This arrangement turns off all the dyes excited by the outer rim of the excitation beam so that collection spot is significantly reduced in size.

We have already accomplished the development of a continuous wave STED microscope with a resolution of 45 nm. We have also explored the property of donut beam focused by high numerical aperture objective and its implications on achievable resolution. In this paper, using home built STED microscopy of 45 nm resolution, we study the self assembly behavior of 190 nm polystyrene particles on various interfaces in high ionic strength media. Our preliminary data obtained on negatively charged glass surface at neutral pH and various ionic strengths show interesting self-assembly behavior. We find that at low ionic strength, particles form gel-like random structures. At high ionic strengths, more organized patterns ranging from 1D linear chain to 2D squares or quasi hexagons are observed. Although not clear, such behavior could originate from the competition between particle-particle and particle-wall interactions. Study on other surfaces, for example, neutral gold film, C18 coated surfaces etc., sheds more light onto the nature of forces involved. We also study the self assembly of rod-like polystyrene nanoparticles. Our study will provide rich information on the self assembly behavior of nanoparticles in situ, which is not available from conventional microscopic techniques.

Keywords: Materials Science, Microspectroscopy
Application Code: Nanotechnology
Methodology Code: Microscopy
Ultra-small semiconductor nanocrystals (SCNCs) bridge the gap between small molecules and quantum dots. Importantly, at ultra-small size the majority of the atoms are on the surface leading to a large number of surface-occupied orbitals available for further hybridization with SCNC’s external environment. Here we demonstrate the stabilization of SCNC’s surface occupied orbitals with organic solvents such as butylamine. The solvent-induced stabilization of surface occupied orbitals has increased the poly(ethylene glycol)-thiolate-stabilized 1.6 nm CdSe nanocrystals core electronic energy up to 140 meV. The increase of core energy was strongly butylamine concentration dependent. Through optical spectroscopy and mass spectrometry analyses, we have proved that the change in core electronic energy is solely due to the delocalization and stabilization of SCNC’s highest occupied (HOMO) and lowest unoccupied molecular orbital (LUMO), not because of core reconstruction. We believe, delocalization and stabilization of surface occupied orbitals by this solvent will increase the radiative lifetime of SCNC and enhance SCNC performance in solid state device applications.
Nanomaterials possess distinctive physicochemical properties and promise a wide range of applications, from advanced technology to leading-edge medicine. However, their effects on living organisms remain largely unknown. In this study, we have designed and utilized various developmental stages of zebrafish embryos as in vivo assays to determine the dependence of biocompatibility and toxicity of nanomaterials upon their physicochemical properties, aiming to effectively predict toxicity of nanomaterials and rationally design biocompatibility nanomaterials for a wide variety of applications. We found that the embryonic developmental phenotypes strikingly depend upon the embryonic developmental stages, and the sizes, doses and chemical properties of nanoparticles (NPs). These notable findings suggest that the NPs are unlike any conventional chemicals or ions. They can potentially enable target specific study and therapy for early embryonic development in size, stage, dose, and exposure-duration dependent manners.

The work is supported in part by NSF (CBET 0507036) and NIH (R01 GM0764401).

Keywords: Bioanalytical, Imaging, Nanotechnology, Toxicology
Application Code: Nanotechnology
Methodology Code: Other (Specify)
Histamine plays a major role in the mediation of allergic reactions such as peripheral inflammation. In addition, histamine is a neurotransmitter involved in the central nervous system, but its roles in this context are poorly understood. Studying histamine neurotransmission is important due to its implication in diseases such as Alzheimer’s and Parkinson’s. The sensitivity, selectivity and high temporal resolution of fast scan cyclic voltammetry (FSCV) offers many advantages for studying electroactive neurotransmitters. Histamine has previously been studied with FSCV (3 refs), however, the lack of a robust Faradaic electrochemical signal makes it difficult to selectively identify histamine in complex media, as found in vivo. In this work, we optimize an electrochemical waveform that provides a stimulation-locked, robust Faradaic signal towards Histamine. We describe in vitro waveform optimization and a novel in vivo model for stimulating histamine release in the mouse substantia nigra pars compacta via stimulation of the tuberomammillary nucleus. We demonstrate that a robust Faradaic signal can be used to effectively identify histamine and characterize its in vivo kinetics.
**Session Title**: Neurochemical Applications of Electrochemistry  
**Abstract Title**: Thin Composite Films for Selective Voltammetric Neurotransmitter Measurements  
**Primary Author**: Richard F. Vreeland  
**University of Arizona**  
**Co-Author(s)**: Christopher W. Atcherley, Levi B. Lazarus, Michael L. Heien  
**Date**: Monday, March 03, 2014 - Afternoo  
**Time**: 01:50 PM  
**Room**: 5505A

**Abstract Text**
Nafion[registered] has been dip-coated onto electrodes to enhance selectivity of carbon-fiber microelectrodes to dopamine, while decreasing the sensitivity to interferents and preventing biofouling. Because Nafion[registered] is a fluoropolymer like PTFE; it does not adhere well to carbon fiber surfaces. A Nafion[registered] and poly(3,4-ethylenedioxythiophene) composite polymer has been synthesized on the cylindrical electrode with the goal of increasing the selectivity and sensitivity of electrochemical dopamine measurement in vivo. Unlike dip-coated Nafion[registered], PEDOT:Nafion coatings are mechanically robust, and can be applied in a controllable and uniform manner. Carbon-fiber microelectrodes have been coated with PEDOT:Nafion via electropolymerization in a fast and facile process, requiring a potentiostat capable of slow-scan voltammetry. Electropolymerization allows any geometry to be coated; the mechanism of the polymerization is described. PEDOT:Nafion coated electrodes are employed in cyclic voltammetric measurements of dopamine and other transmitter molecules. The sensitivity and selectivity of the coated electrodes are characterized and compared to bare carbon fibers, and carbon fibers dipped in a Nafion[registered] solution. Electrodes are coated with a minimally thin film of approximately 100 nm to reduce unwanted diffusive impedance of the analyte to the electrode. Furthermore, PEDOT:Nafion electrodes have been characterized by scanning electron microscopy, energy-dispersive x-ray spectroscopy, and electrochemistry. These coatings are shown to contain sulfur and fluorine, and enhance the sensitivity and selectivity of cation measurement, while reducing the signal from anions.

**Keywords**: Bioanalytical, Biosensors, Electrochemistry, Polymers & Plastics  
**Application Code**: Bioanalytical  
**Methodology Code**: Electrochemistry
Carbon-fiber microelectrodes (CFMEs) are excellent sensors for in vivo monitoring of chemical communication due to their minimally invasive geometry, excellent electron transfer properties, and biocompatibility. Furthermore, several biogenic amines are known to adsorb to carbon. This is exploited to enhance sensitivity of fast-scan cyclic voltammetry, which is often employed with CFMEs. Electrodes are traditionally prepared using commercially available carbon fibers, which are fabricated with a limited variety of radii. Additionally, the fragility of small fibers complicates electrode fabrication. Differences in fiber manufacturing processes and the resultant variation in morphology and surface chemistry make comparative analysis across electrodes based solely on electrode radius challenging. In this work we reduce the radii of commercially available carbon fibers using microwave-induced air plasma to etch CFMEs. This allows for controllable fabrication of identical electrodes. SEM analysis shows a decreased electrode radius with increased etching time and the etch rate was measured to be 0.3 [micro]m/minute. Fast-scan cyclic voltammetry is used to quantify electrode sensitivity to dopamine and other neurotransmitters after etching. Surface properties (adsorption dynamics and electron-transfer kinetics) compare favorably with native carbon-fiber microelectrodes. Mass-transport limited electrode response time is quantified using fast-scan controlled-adsorption voltammetry. Mechanical stability was validated with experiments in agarose gels, which serves to mimic the consistency of the brain. Plasma etching of electrodes provides a way to fabricate electrodes with tunable radii to reduce the electrode response time. These factors affect reproducibility and sensitivity of in vivo electrochemical measurements.

Abstract Text

Biosensors, Electrochemistry, Microelectrode, Neurochemistry

Application Code: Neurochemistry

Methodology Code: Electrochemistry
Neuronal activity elicits changes in local cerebral blood flow (CBF) that replenishes metabolic substrates and clears waste, a relationship known as functional hyperemia. Better understanding functional hyperemia would increase understanding of neurological pathologies linked with poor CBF regulation. While many methods exist for monitoring CBF changes, all have limitations, including poor spatial/temporal resolution, the inability to measure blood flow beneath the cortex, and the requirement that subjects stay motionless. To overcome these shortcomings, we have produced microfabricated sensors utilizing electrochemical hydrogen clearance (EHC).

EHC involves two stages: First, an electrochemically generated pulse of hydrogen saturates the tissue around an electrode. Then hydrogen clears from the area through a combination of blood flow and diffusion. The clearance rate, from which CBF is calculated, is monitored amperometrically at a second electrode. EHC has been used to quantify blood flow in many tissues, and hydrogen clearance without electrochemical generation has been used to monitor CBF in freely-moving animals. Previously, the large size of EHC electrodes has limited its use in the brain. Microfabrication mitigates the problem, yielding reproducible micrometric devices that limit tissue damage and function with high spatio-temporal resolution.

We present microfabricated devices capable of EHC CBF measurements. The devices are photolithographically patterned onto silicon substrates and coated with evaporated platinum metal. We compare experimental hydrogen collection efficiencies to theoretical models \[\textit{in vitro}\]. Results that compare the \[\textit{in vitro}\] use of these microelectrodes under different conditions are presented, emphasizing selectivity and longevity in conditions mimicking the brain.

Funding from the NIH/NIDA (DA032530).

Keywords: Electrochemistry, Microelectrode, Neurochemistry, Sensors
Application Code: Neurochemistry
Methodology Code: Electrochemistry
During chemical communication neurotransmitters are released into the extracellular space and then diffuse across the synapse. Some neurotransmitters leave the synapse and can bind to receptors that are farther away (volume neurotransmitters). Thus it is important to understand how mass transport is altered under conditions of neuronal inflammation and disease. Fast-scan controlled-adsorption voltammetry (FSCAV) is a technique recently developed that exploits adsorption processes at carbon-fiber microelectrodes, enabling direct measurements of absolute concentrations. A finite element simulation was developed to aid in quantifying the dynamics of mass-transport-limited adsorption. FSCAV utilizes a digital switch to control adsorption by allowing different waveforms to be applied to the electrode with fine control. Specifically by altering the waveform application frequency, the strength of adsorption is changed. This allows us to use fast-scan cyclic voltammetry for absolute concentration measurements, which is advantageous because the shape of the cyclic voltammogram is the primary factor used for analyte identification. Here we present a data processing strategy to confer selectivity for dopamine and serotonin over metabolites in vivo. The diffusion coefficients for dopamine and serotonin were determined in the nucleus accumbens and substantia nigra respectively.

Keywords: Bioanalytical, Electrochemistry, Microelectrode, Neurochemistry
Application Code: Biomedical
Methodology Code: Electrochemistry
Comparison of Novel Metal and Novel Carbon Based Electrodes for Use in Online Microfluidic Neurochemical Detectors for Microdialysis

Real-time measurement of the neurochemical changes associated with adverse events is proving a powerful tool in the clinical management of traumatic brain injury patients. Clinically the neurochemicals are sampled using microdialysis to achieve high time resolution measurement of the dialysis stream required high performance sensors and biosensors that function well within a low-volume microfluidic chip. Traditionally such electrodes are made of gold or platinum. These are easy to microfabricate but their performance often degrades quickly when exposed to biological samples. In this abstract we compare the performance of electrochemical sensors and biosensors made from novel carbon-based materials such as graphene and single-walled carbon nanotubes with platinum based electrode for a range of important neurochemical metabolites and neurotransmitters.

Bioanalytical, Electrochemistry, Lab-on-a-Chip/Microfluidics, Neurochemistry

Neurochemistry

Electrochemistry
Deep eutectic solvents (DES) are considered as alternative green solvents because of their physical, chemical, thermal properties, environmental, and economical benefits. They are now of growing interest in many fields of research including catalysis, organic synthesis, electrochemistry, biotransformation, dissolution, and extraction processes. DES are eutectic mixtures formed between quaternary ammonium salts (QAS) and hydrogen bond donors (HBD). A variety of DES based on choline chloride,-and acetyl choline chloride QAS and urea,-and glycerol HBD were synthesized by altering the composition and molar ratio. Their solubility as solvents for lignocellulosic biomass was evaluated by dissolving various biomass components such as sugars, cellulose, hemicellulose, lignin, starch, and protein. Most of the selected DES showed high lignin solubility and very poor or negligible solubility for rest of the biomass components checked. Therefore the selective extraction of lignin from prairie cordgrass (PCG) and switchgrass (SWG) with DES was carried out by optimizing the extraction temperatures, extraction time, and the acid content. Extracted lignin was confirmed by fourier-transform infrared (FTIR) spectroscopy, matrix-assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-TOF-MS) and proton nuclear magnetic resonance spectroscopy (1H NMR). Recovery and recycling of DES was carried out by vacuum distillation, recrystallization and the composition of the recovered DES was measured by 1H NMR.
The relationship between retention and temperature for a series of analytes on a lauryl acrylate porous polymer monolith (PPM) stationary phase was investigated with capillary electrochromatography (CEC). Columns are prepared in-house in fused silica capillaries with 75 micron inner diameters. Polymerization is done in situ and the resulting columns are reproducible, rugged, and efficient. Columns tend to break before separations degrade. An alkyl benzene series (toluene through octyl benzene) was used to probe the thermodynamics of retention over the range of 25 degrees C to 60 degrees C in 5 degree C increments. Analytes were selected based on their use in our previous studies and their inclusion in other literature reports. The van’t Hoff relationship: \[ \ln k = \frac{-H}{RT} + \frac{S}{R} + \ln \phi \] where \( k \) is the retention factor, \( H \) and \( S \) are respectively the enthalpy and entropy of partitioning, \( \phi \) is the phase ratio (volume of stationary phase/volume of mobile phase), and \( R \) and \( T \) are respectively the gas constant and temperature describes this relationship. The enthalpy of partitioning (\( H \)) is obtained from the slope and the entropy of partitioning (\( S \)) is obtained from the y-intercept, provided the phase ratio (\( \phi \)) is known. The phase ratio can be reliably obtained using a nanoflow HPLC instrument because our column preparation does not require the placement of frits in the capillary. Accurate volume measurements can be measured and compared for a polymer filled column and a capillary of the same length but filled only with mobile phase. System volumes are also easily measured by by-passing the column. \( \phi \) for these columns was determined to be 0.189 by HPLC. Both \( H \) and \( S \) were found to decrease as retention increases. Since \( G \) also decreases as retention increases, retention on the lauryl acrylate PPM is concluded to be enthalpically driven. Values were compared to published values for columns utilizing more traditional stationary phases. These comparisons revealed a less steep decrease in the \( H \) trend for the PPMs. Diffusion studies are currently being conducted across a temperature range to investigate the thermodynamics of adsorption, desorption, and diffusion mechanisms of analytes in the presence of lauryl acrylate PPM. This work is supported by the National Science Foundation, the American Chemical Society Project SEED and Petroleum Research Fund, the Welch Foundation, and the Howard Hughes Medical Institute, and the McNair Scholars Program.
Abstract Text

Essential oils are concentrated hydrophobic aroma compounds from plants. They are used in consumer’s goods from food, pharmaceuticals and cosmetics industries. Conventional processes for essential oil recovery involve steam distillation and organic solvent extraction. Steam distillation involves high heat, which can cause sample hydrolysis and thermal degradation of heat sensitive compounds whereas in organic solvent extraction, polluting organic solvents and expensive post-processing of the extract for solvent elimination is involved. Supercritical fluid extraction (SFE) has immediate advantages over these traditional methods and offers alternative extraction method. It is a flexible process due to the possibility of modulation of solvent power by changing pressure and temperature. Supercritical fluid carbon dioxide (SF-CO2) was used to extract essential oil from chrysothamnus nauseosus (rabbit brush) and rhus aromatica (skunk brush), which are native to western North America and both, have traditional medicinal use. The effect of vital parameters on extraction efficiency, pressure, temperature and static and dynamic periods were investigated in the range of 1000-7000 psi, 304-323 K, 10-40 min and 10-30 min respectively. GC-MS and GC-FID were utilized in the identification and quantification of essential constituents. Maximum extraction yield was obtained at optimum conditions of 5000 psi, 37°C, and 15 min (static) and 30 min (dynamic) times. The main compounds obtained were β-phenylcarbinol, β-pinene, linalool, γ-curcumene, isopryrethrone, and spathulenol for rabbit brush and limonene, β-cubebene, and β-tocopherol for skunk brush.

Keywords: Extraction, Optimization, Volatile Organic Compounds
Application Code: General Interest
Methodology Code: Separation Sciences
To expand the application of the refractive index (RI) sensing modality, we apply microring resonator arrays as a universal detector for HPLC. RI-based sensors offer attractive characteristics as non-destructive and universal detectors, but a small dynamic range and sensitivity to minor thermal perturbations limit the use of commercial RI detectors in chromatographic applications. As such, RI detectors find use in sample abundant, isocratic separations when interfaced with HPLC. Silicon photonic microring resonator arrays are RI-based sensors that can track large fluctuations in refractive index and operate under ambient conditions. The increased dynamic range enables operation of the sensor across a wide spectrum of refractive indices, such as moving from an aqueous to organic mobile phase. Mobile phase gradients are reproducible independent of temperature fluctuations and enable gradient separations using an RI sensing modality unsupported in commercial devices. Additionally, thermal controls are integrated into the array eliminating the need to operate under tightly controlled thermal conditions, obviating extended detector equilibration wait times. Consequently, microring arrays avoid the major pitfalls of RI-based detection while offering bulk RI sensitivity comparable to conventional detectors. Previous applications of silicon photonic sensors utilized the enhanced surface sensitivity of the device by differentially functionalizing the sensor surface, which preferentially interacts with analyte(s) of interest. Similarly, the sensor array surface could be modified in HPLC applications to enhance sensitivity or to perform 2-D separations (e.g., silane functionalization, antibody immobilization), and since the array has a small footprint (4 mm x 6 mm), RI detection has potential for integration into lab-on-a-chip devices.

Support for this work was provided by the National Science Foundation Graduate Research Fellowship Program.
In pursuit of wider selectivity envelopes and increased retention for Supercritical Fluid Chromatography (SFC) separations, we have developed a series of new bonding chemistries. These next generation SFC stationary phases are based upon functionalizing hybrid particles with novel bondings containing judiciously chosen selectors, which significantly improve peak shapes, reducing the need for mobile phase additives. In addition, these bondings significantly increase retention and limit unwanted interaction between analytes, mobile phase and the base particle. Such interactions have led to observed retention losses of >15% over several weeks for the previous generation of chromatographic materials, significantly reducing reproducibility in SFC separations. In the past, this was attributed to instrumentation, but high performance SFC systems have shed new light on the source of this variation. Our next generation SFC stationary phases effectively eliminate these retention losses. Over a twenty day period, a 2-ethylpyridine bonded hybrid material had an average retention loss of 13.2% for five probes (Fig. 1). This was reduced to a 0.3% average loss in retention for the new materials over a similar time frame. By reducing retention variance over time and maximizing selectivity with simple co-solvents, our new generation of stationary phases will facilitate robust analytical methods. Attributes and mechanisms of these novel stationary phases will be discussed and explained regarding selectivity, retention and peak shape for basic compounds.
Convergence chromatography (CC) is a separation technique that utilizes compressed carbon dioxide (CO\textsubscript{2}) as the primary mobile phase component to achieve unique selectivity, low solvent usage and high efficiencies. The use of supercritical fluid as a mobile phase provides higher diffusivity and lower viscosity than liquid mobile phases, thereby providing higher throughput and chromatographic efficiencies as compared to liquid chromatography.

Convergence Chromatography provides high efficiency separations which are beneficial for the analysis of complex samples, such as natural products. However, identification and confirmation of the numerous components found in natural products can be challenging. To address this challenge by LC, multiple detectors are typically used during a single analysis whereas each detection technique is based on a different physical or chemical property of the molecule. For example, mass detectors and PDA are commonly combined to obtain both mass and UV-spectral information. Evaporative light scattering, a more universal technique, addresses compounds without ultraviolet absorbance (no chromophore) and poor ionization by MS. The combination of these three detection techniques allows for analysis of a wide range of compounds. In this presentation, we will investigate the analysis of a number of natural products using triple detection in combination with CC. Identification and quantitation of compounds will also be illustrated. Guidance combining triple detection with CC will be provided based on the observations attained throughout the analysis. This approach when combined with sub-2 µm column chemistries will allow for the analysis of a wide range of compounds by convergence chromatography.

Keywords: Light Scattering, Mass Spectrometry, Natural Products, SFC
Application Code: General Interest
Methodology Code: Separation Sciences
Distiller’s dried grain with solubles (DDGS) is a byproduct during the ethanol production process from corn that is exclusively used as a livestock feed. It contains low starch and high levels of protein, fiber, minerals and vitamins, making it ideal as a diet to treat medical conditions like diabetes and celiac disease in human beings. The presence fatty acids create an off flavor while the dark color affects the appearance of baked products limiting its utilization. Use of supercritical carbon dioxide as a potential bleaching technique was investigated to modify odor and color in order to make flours with enhanced baking functionality. Parameters such as pressure, temperature, flow rate, particle size, and extraction time were optimized and the nutritional content of bleached DDGS was determined.

Keywords: Extraction, Separation Sciences, SFE
Application Code: Food Science
Methodology Code: Separation Sciences
Helium is a common inert gas, but for commercial purposes it’s usually generated as a byproduct from natural gas mining. Because recession has caused a slowdown in natural-gas production, helium markets are facing a shortage; more plants will coming online by the end of 2012 in some countries but not in time to ease the current crisis to avoid lack of helium like it occurs in 2012 in European countries where many laboratories have faced for several weeks in a non-helium supply.

Three gases are commonly used as carriers in gas chromatography: nitrogen, hydrogen, and helium. While nitrogen provides higher chromatographic efficiency than hydrogen or helium, the main consideration is to obtain the required separation in the short time. There is not a great difference between helium or hydrogen in terms of diffusivity, but hydrogen has about half the viscosity of helium and nitrogen; therefore, when hydrogen is used the best separation is achieved faster allowing reduction of run time.

When we think about cylinders, hydrogen is potentially explosive and care must be taken when it is used as carrier gas in the case gas chromatography mass spectrometry. When a hydrogen generator is used with a control probe implanted inside the GC oven the dangers associated with leaks and subsequent accumulation is banish thanks to an auto stop of the hydrogen generators as soon as a leak is detected and GC–MS systems are hydrogen amenable with appropriate precautions.

In order to replace helium by hydrogen in our laboratories, a long term study was setting up to evaluate the performance and the drawback of hydrogen as a carrier gas for the analysis of organophosphorous pesticides by gas chromatography–mass spectrometry with electron ionization. Several parameters were explored as the effect of the diffusivity of hydrogen on a vacuum system, the eventual variation of the fragmentation pattern of the steroids and the change on detection capacity of the system.

Keywords: Environmental Analysis, Environmental/Waste/Sludge, Gas Chromatography/Mass Spectrometry, Th Application Code: Environmental Methodology Code: Gas Chromatography/Mass Spectrometry
Not all analytical measurements and clinical diagnoses need to be expensive tests performed in a laboratory setting. Sometimes, bringing the lab to the sample is the best solution. Paper-based analytical devices (PADs) provide the opportunity for inexpensive yet accurate in-field testing. These are particularly useful in areas where resources are limited. The research developed novel chemical assays with environmental applications. These tests were colorimetric and the color changes in the presence of nanoparticles will be measured and evaluated. The goal of this research was to develop inexpensive, nanoparticle-based colorimetric tests on paper-based microfluidic devices. The steps of the project were 1) synthesize and characterize nanoparticles 2) develop both novel solution-phase assays and also implement those based on chemical literature 3) fabricate and optimize paper-based microfluidic devices for assays. We have developed three types of assays based on different functionalization methods: Ca\[^{2+}\], Sr\[^{2+}\], Ba\[^{2+}\]; Al\[^{3+}\]; and Fe\[^{3+}\]. We characterized the nanoparticles by visual observation of the color change and by UV-VIS absorbance spectroscopy. The colorimetric changes were also characterized by UV-VIS and with a digital camera. The color changes on paper-based devices were characterized by capturing a digital image with a scanner. These tests can eventually be applied to development of a range of metal ion tests to be used in on-site water quality testing.

Keywords: Analysis, Environmental/Water, Lab-on-a-Chip/Microfluidics, Nanotechnology

Application Code: Environmental
Methodology Code: Chemical Methods
Rice husk is a by-product of rice milling process and abundantly available waste material in all rice producing countries. Upon calcination, rice husk produced of about 20% ash of which 90% was silica. Acid treatment of rice husk prior to calcination was proved to be very effective in producing silica with high purity. In this experiment, rice husk was treated using hydrochloric acid and citric acid for the metal impurities removal. Thermal treatment was conducted at 750°C and maintained for 5 hours. Silica was mostly deposited on the epidermal layer and showed similarity with the intact structure of rice husk based on SEM investigation. The resulted silica was proved to be amorphous according to FTIR (Fourier Transform Infrared Spectroscopy) and XRD (X-Ray Diffraction) analysis. The BET (Brunauer-Emmett-Teller) specific surface areas of silica derived from calcined hydrochloric acid treated and citric acid treated rice husks were about 312 and 255 m²/g, respectively. Silica with purity of about 99% was obtained in all calcined acid-leached rice husks based on XRF (X-ray Fluorescence) analysis. Citric acid treatment was less harmful and less corrosive as compared to hydrochloric acid and was still effective enough in producing high-grade silica with high purity and high surface area. Pure amorphous silica derived from rice husk had a great potential to be used as adsorbents or catalyst supports or even as low-cost precursors for other silica based products.

Research reported in this publication was supported by grant number III/LPPM/2012-09/80-P from the Institute for Research and Community Services of Parahyangan Catholic University, Bandung, Indonesia.

Keywords: FTIR, Materials Characterization, X-ray Diffraction, X-ray Fluorescence
Application Code: Materials Science
Methodology Code: X-ray Techniques
1-Methylcyclopropene (1-MCP) is an inhibitor of the plant hormone ethylene and has commercial applications in cut flowers and stored fruits and vegetables. Under ambient conditions, 1-MCP exists as a gas and functions by tightly binding to ethylene receptors and preventing normal regulatory processes such as ripening. In this study, the extent of 1-MCP absorption was investigated for cardboard packaging material and bananas at different stages of ripeness upon exposure in an air-tight chamber. Samples of the chamber headspace were analyzed over time by GC-FID and GC-MS. Additionally, the production of banana volatiles were monitored over time with treated and untreated fruit. Results show that cardboard packaging absorbs a significant amount of 1-MCP but at a slower rate than bananas, and that exposure to 1-MCP markedly reduced the evolution of selected volatiles in bananas.

Support for this project was provided by AgroFresh, Inc., Spring House, PA 19477.
Carbon paste microelectrodes electrodes are easily fabricated, inexpensive and can be used with electroanalytical-based microfluidic devices. In this work, the electrochemiluminescent (ECL) reaction between tris(2,2'-bipyridyl)ruthenium(II) and biogenic amines was used to detect these amines in a microfluidic flow system. An ECL reaction was observed for the following amines in the microfluidic flow system: spermine, spermidine, and putrescine. The ability to measure these biogenic amines is important for food safety applications. Their response was compared to tri-propylamine, a well-characterized ECL participant. The flow system parameters optimized included the applied potential, flow rate, and electrode fabrication method. The response was linear over a concentration range of 10–100 micro-M with a limit of detection (S/N = 3) of 1.8 micro-M for spermine, a linear range of 10–100 micro-M and a LOD of 4.3 micro-M for spermidine, and a linear range of 35–125 micro-M and a LOD of 28 micro-M for putrescine.
Carbon paste electrodes (CPEs) are easily fabricated, disposable, and can be used in conjunction with microfluidic devices to fabricate inexpensive biosensors. In this work, a screen printed-like carbon paste microelectrode was coupled to a microfluidic flow system. The electrogenerated chemiluminescent reaction between hydrogen peroxide and luminol generated the analytical signal. The biosensor fabrication method was optimized, along with key experimental parameters (applied voltage, solution flow rate, buffer species and luminol concentration). A new fabrication method involved adding poly(dimethyl)siloxane to the carbon paste mixture and resulted in more sensitive and robust electrodes. A typical RSD for the response of the optimized electrodes was ~3%. The limit of detection (LOD) for hydrogen peroxide using this new fabrication method was 50 micro-M. The electrodes gave a linear response between 50 micro-M and 200 micro-M hydrogen peroxide. This range could be extended by using a higher concentration of luminol. Electrodes with glucose oxidase (GOx) were fabricated using this new method, and optimal GOx concentrations and electrode stability were investigated. Glucose could be indirectly detected via of hydrogen peroxide by the enzymatic reaction at the biosensor.

Keywords: Biosensors, Chemiluminescence, Electrochemistry, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Analytical instruments have been used to study human odor and validate dog-tracking evidence for the judicial community. It is believed that people continuously shed epithelial cells, leaving a trail as they move about. These cells can degrade, giving off Volatile Organic Compounds (VOCs) that create a unique scent for dogs to follow. Our goal was to investigate if these scents are differentiable and stable over time. Twenty samples collected from unwashed hands of people of different genders, ages, and ethnic backgrounds, were studied. Samples were sealed in glass jars and allowed to degrade for 12-18 hours to fill the headspace with VOCs. Headspace preconcentration and GC/MS were used to analyze the resulting VOCs. Principal Component Analysis (PCA) was used to evaluate potential class characteristics and differentiability within the twenty subjects. With a limited sample pool, no obvious trends were observed among different genders and ethnic groups, but college age and non-college age appeared to have some separation. Two different time studies were also conducted. In one study, seven volunteers were sampled every week over four consecutive weeks to examine how a person’s scent changes over time. Over the four week period, the composition of human scent from the same person appeared to change somewhat. In the other study, six samples were collected from the same volunteer, left exposed to air, and run after 1 day, 2 days, 4 days, 1 week, 2 weeks, and 3 weeks to see how stable a collected sample is over time.

**Keywords:** Forensic Chemistry, GC-MS, Mass Spectrometry, Volatile Organic Compounds

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Mass Spectrometry
This research project is a collaboration between several institutions across the country in which mass spectrometry and other proteomic techniques are used to identify proteins from Tetrahymena thermophila. Tetrahymena thermophila is a freshwater ciliate protozoan used as a model organism to provide insight into the workings of other organisms. Protein samples acquired from SDS-PAGE gel electrophoresis were separated into their individual bands and further cut to increase surface area. The disulfide bonds of the proteins were reduced and alkylated, and the proteins were then digested with trypsin. The tryptic peptides were removed from the gel and then purified with C18 pipette tips. Samples were then analyzed with a MALDI-TOF/TOF mass spectrometer and the resulting mass spectra were compared to the NCBInr protein database using the MASCOT search program. Individual peptides chosen from the mass spectra were then further fragmented to obtain sequence information and confirm the primary sequence of the peptide. Out of 29 gel bands analyzed from Tetrahymena thermophila samples, 10 proteins previously not seen in these samples were identified, including 7 of unknown function. In addition, 31 proteins that had previously been identified were confirmed to be present.

Keywords: Mass Spectrometry, Proteomics, Tandem Mass Spec
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Mass Spectrometry
Protein conformational heterogeneity, motion, and flexibility are thought to significantly impact protein function, but investigation of their contribution is limited by the availability of methods for characterizing rapidly fluctuating protein states. Infrared (IR) spectroscopy can be employed to directly probe the structural dynamics of proteins on fast timescales. However, the spectral congestion inherent to protein spectra limits its application to the study of protein systems. To alleviate this issue, we incorporated into proteins vibrational probes with spectrally isolated frequencies that make possible rigorous analysis of protein environments and dynamics with IR spectroscopy. In particular, heme-bound carbon monoxide and selectively incorporated cyano amino acids were introduced as probes of cytochrome P450. These were then used with linear and two-dimensional IR spectroscopy to investigate the contribution of protein dynamics and conformational heterogeneity to the specificity of cytochrome P450 catalytic activity. In addition, further studies of mutants of cytochrome P450 were performed to investigate the specific protein residues that contribute to the protein's dynamics.
Sonodynamic therapy (SDT) is a promising cancer treatment modality which combines a sonosensitizing drug and oxygen with high-frequency ultrasound in the megaherz range to cause selective damage of the target tumor tissues. Despite remarkable laboratory results reported in the field of SDT over the years, its clinical utility has largely been hindered due to the fact that most sonosensitizers suffer from limitations such as significant toxicity, relatively low tumor affinity, limited sonosensitivity, and low clearance rate from normal tissues. In this study, we systematically integrated normal chitosan nanoparticles with three additional functions, namely targeting moieties (antibodies specific to particular cancer cell receptors of interest), imaging tags (fluorescent dye such as caboxyfluorecein) and therapeutic reagents (sonosensitizer). A variety of sonosensitizers (e.g., chlorin-e6, emodin, methylene blue, and rose bengal derivatives) were investigated for their production of reactive oxygen species after exposure to different doses of ultrasound irradiation. It is anticipated that noticeable difference would be found in terms of their sonodynamic efficacy in vitro and in vivo, following the introduction of various formulations of multifunctional chitosan nanoparticles.

Keywords: Drug Discovery, Fluorescence, Materials Science, Molecular Spectroscopy

Application Code: Nanotechnology

Methodology Code: Molecular Spectroscopy
Hydrophilic Interaction HPLC Determination of Creatinine, Urate and Ascorbic Acid in Bovine Milk and Orange Juice

Creatinine, uric acid (UA) and ascorbic acid (AA) are common components in human fluids. Their abnormal concentrations in human fluids are associated with various diseases, such as gouty arthritis, hyperuricemia, hypertension, pneumonia and kidney damage. Thus, besides their endogenous production in human body, it is also important to examine their sources from food products. In this study, a rapid and accurate hydrophilic interaction HPLC method was developed for simultaneous determination of creatinine, UA and AA in bovine milk. Milk samples were pretreated by protein precipitation, centrifugation and filtration, followed by HPLC separation and quantification using a Waters Spherisorb S5NH2 column and a UV detector with an isocratic elution containing acetonitrile and phosphate buffer at pH 4.75. The developed HPLC method has been successfully applied to determine the concentration of UA, AA and creatinine in milk and fruit juice samples. The milk samples tested were found to contain UA and creatinine in the concentration range of 24.1-86.0 and 5.07-11.15 µg/mL, respectively. The orange juices contain AA over 212 µg/mL.

Keywords: Agricultural, Beverage, Biological Samples, HPLC
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
### Abstract Text

Diversification of our country’s science talent pool is critically needed and can only be achieved by stimulating interest in STEM among students from a wide variety of cultural backgrounds. However, motivating, increasing the number, improving retention rates and graduation rates of underrepresented minority (URM) students in STEM disciplines continue to be a major challenge to Historically Black Colleges and University (HBCU). Early involvement of URM students in research is a viable strategy to excite minority students in STEM areas. The outcome and the results of the use of the Raising Achievement in Mathematics and Science (RAMS) and Summer Undergraduate Research Experience (SURE) programs at Winston Salem State University (WSSU) as a strategy for stimulating the interest of URM students in STEM education at a HBCU institution will be discussed and presented. A representative of the SURE project involving the determination of moisture content of pharmaceuticals and powdered foods using Karl Fisher Titration method in a guided inquiry (GI) setting will also be presented. The influence of the RAMS and SURE programs on the retention rate and STEM education was examined. Furthermore, the experience of RAMS scholars and SURE participants was evaluated by administering a survey to the participants after the completion of the program. The retention rates of the RAMS scholars and SURE participants were considerably better than non-RAMS scholars. The result of the analysis of the survey indicated that the RAMS and SURE program overtly generated URM student excitement, while promoting critical thinking and teamwork. Moreover, RAMS scholars and SURE participants particularly enjoyed other program enrichment activities, including professional development seminars and social activities as well as poster and oral presentation at regional and national conferences.

### Keywords:
- Education
- Electrochemistry
- Pharmaceutical

### Application Code:
- Pharmaceutical

### Methodology Code:
- Education/Teaching
Gas Chromatography

Characterizing the Performance of Surface Modifications that Enhance Sensitivity, Reliability, Reproducibility and Accuracy of Analytical Instruments

Analytical and process testing systems are often constructed of materials, such as stainless steel or glass that can contribute to poor reproducibility and inaccurate analyses. The mechanism of failure can result from a variety of factors such as chemical species adsorption to system substrates, attack from corrosive media resulting in metal ion contamination, and/or catalytic interaction with metal ions such as nickel, chromium or iron. The application of a thin, inert, protective coating that does not alter the mechanical tolerances of the original system design would be highly beneficial in eliminating testing system inaccuracies as well as extending serviceable lifetimes.

A novel carboxysilane material has been developed and used within several industrial and analytical fields for over two years. This material is deposited via Chemical Vapor Deposition on 3-dimensional stainless steel and glass substrates, and within extended lengths of tubing (up to 2000’ long). This diffusion coating exhibits substantial improvements in inertness, hydrophobicity, corrosion resistance, and wear resistance over the underlying substrate. Examples of components that benefit from this coating include valves, tubing and fittings, and gas and fluid transfer systems. Several surface characterization techniques are used to define the material specifications.

Inertness testing data includes recovery and breakdown component testing of active compounds. Anti-corrosive performance for acid, base and saltwater exposure will be evaluated via ASTM and EIS methods. Comparative pin on disc wear resistance data of the carboxysilane material will demonstrate a significant improvement in wear resistance over 300 series stainless steels.

Keywords: Biopharmaceutical, GC, Petrochemical, Pharmaceutical
Application Code: Bioanalytical
Methodology Code: Gas Chromatography
Stability and linearity testing is imperative to providing customers with the highest quality gas mixtures. These types of studies ensure the equipment is operating properly, and provides the necessary information to determine the measurement accuracy of the equipment. The method of analysis was a Varian 4900 Micro Gas Chromatograph equipped with a Thermal Conductivity Detector (TCD) with a molecular sieve 5A column. A 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 percent level methane balance argon mixtures; it was validated by completing a Gage R&R study, a linearity study, a repeatability study, along with continuous long term stability monitoring. The studies concluded the system was linear, the data can be repeated, and the data can be reproduced over a range of days and operators. The LTS monitor has demonstrated that over time the system has remained stable for the measurement of methane in argon gas mixtures.

Keywords: Analysis, Chromatography, Data Analysis, GC Detectors
Application Code: General Interest
Methodology Code: Gas Chromatography/Mass Spectrometry
Initial strides toward a microanalytical system capable of comprehensive two dimensional gas chromatographic separations (GC × GC) are presented. The core of the system is a two-stage microthermal modulator (µTM) chip (13 × 6 × 0.5 mm) which consists of two interconnected etched-Si microchannels (4.2 and 2.8 cm long) with a cross-section of 250×140 µm, an anodically bonded Pyrex cap, and a 0.3-µm-thick crosslinked wall coating of PDMS. On-chip heaters and sensors allow heating rates up to 2400 °C/s, with adjustable heating pulse width, maximum temperature, stage delay, and modulation period. The µTM is mounted atop a thermoelectric cooler that allows rapid cooling to -20 ºC during each modulation. This cryogen-free µTM draws only 10 W for heating and 21 W for cooling. Previous reports with commercial first- and second-dimension capillary columns yielded excellent results. Here we report on the integration of first- and second-dimension microfabricated columns (µcolumns); a significant step toward a GC × GC system. We used a series of two etched-Si µcolumns (3 × 3 cm chips; 250×140 µm c. s.; 3-m length per chip, wall-coated PDMS) as the first dimension, and a single etched-Si µcolumn (1.2 × 1.2 cm chip; 150×50 µm c. s.; 0.5-m length) with a trigonal tricationic room-temperature ionic liquid (RTIL) stationary phase as the second dimension. The RTIL was stable in air up to 385 °C (TGA), showed a phase transition at -27 °C (DSC), and gave 1,700 plates/m (Golay plot) using napthalene as the probe. A structured 17-component GC×GC chromatogram was generated in < 10 min. Temperature-ramped operation increased peak heights up to 2-fold, while maintaining resolution and modulation ratios similar to those obtained isothermally. The strong retention polar analytes by the RTIL phase leads to inordinately broad second dimension peaks (see figure) compared to a commercial capillary with the same phase. This is being mitigated by decreasing the RTIL film thickness.
Gas Chromatography

Recent Advances to Ensure Simple, Leak Free GC Column Connections

While one of the most basic steps in chromatography, proper column installation can make or break GC and GC/MS system performance and productivity. Forming and maintaining leak free column connections are critical to achieve low background noise, sharp peaks on active compounds, and optimal column resolution and lifetime. Less frequent detector maintenance is another benefit of leak free column connections. A variety of products are available to install fused silica GC columns. Recent advances in capillary column ferrule and column nut design improve the likelihood of sustaining leak free seals. To get the most of GC and GC/MS systems, a review of the current options available to install GC columns is warranted. The selection of capillary ferrule materials offers pro’s and con’s which may be related to the application. While easy to seal because they are soft, graphite ferrules are porous making them unsuitable for use in some detectors, including mass spectrometers. Graphite/Vespel composite ferrules are compatible with mass spec, but shrink at high temperatures creating leaks if used with standard column nuts. Both these traditional material ferrules can negatively impact chromatographic results. Metal ferrules, including flexible metal ferrules, are stainless steel providing unique advantages over other ferrules, but require care with regard to possible over-tightening and fitting damage. To ensure robust and reliable seals, the proper column ferrule for the application is combined with a column nut that both properly seats and secures the ferrule during operation. A new column nut design that extends use of composite ferrules by addressing material limitations is reviewed. Tips for ensuring proper placement of the column into the inlet or detector fitting for consistent sample transfer is also discussed.

Abstract Text

Co-Author(s)  Lindy Miller, Ponna Pa

Abstract Text

Keywords: Environmental/Waste/Sludge, Forensic Chemistry, Gas Chromatography/Mass Spectrometry, GC Col
Application Code: Other (Specify)
Methodology Code: Gas Chromatography/Mass Spectrometry
There is much recent effort focused on the miniaturization of bench-top instruments for field use. Ideally, such instruments would be small, low power, and low cost. Micro gas chromatography columns have followed a similar trend, but are limited by the high cost associated with fabrication. The objective of this work was to develop an all polymer microcolumn for gas or vapor separation utilizing polymer processing methods, which would yield a device less expensive and more suitable to mass manufacture than micromachined and coated microcolumns. Here, we present the fabrication and performance of a thermoset polymer microcolumn for gas separation. The fabrication method described utilizes a reusable micromachined plastic mold to cast and cure a thermoset polymer which acts as both the structural material and stationary phase of the microcolumn, obviating the need for lithographic etching and a thin-film coating step. The fabricated microcolumns are tested using an HP5890 Series II GC/FID system with helium as the mobile phase. Gas separation is demonstrated for mixtures of VOCs with a theoretical plate count of >1500. Surface characterization is explored as a means to understand the influence of polymer morphology on separation performance.

This research was supported by the Department of Defense and National Science Foundation, and carried out in part in the Frederick Seitz Materials Research Laboratory Central Facilities, University of Illinois.
Polycyclic Aromatic Hydrocarbons (PAHs) are formed from incomplete burning of carbon containing fuel. There are thousands of PAH compounds in the environment and of those; there are several that have been established to be of concern for the environment. Extraction of PAH compounds involves a large amount of sample and solvent, and because of this, there is a lot of solvent waste. The use of Large Volume Injection (LVI) in conjunction with a Programmable Temperature Vaporizer (PTV) aids in eliminating some of this solvent waste due to the ability to inject larger volumes of sample without sacrificing sensitivity. This analysis will compare PAH compound response of a standard injection versus a large volume injection.

Keywords: Environmental, Gas Chromatography, Sample Introduction, Semi-Volatiles
Application Code: Environmental
Methodology Code: Gas Chromatography
Although several reports of microscale gas chromatographic (GC) instrumentation employing microsensor array detectors have appeared, the chemometric analysis of the data generated has not been addressed sufficiently. Here, the first application of a multivariate curve resolution method that combines evolving factor analysis (EFA) with alternating least squares (ALS) to partially co-eluting vapors measured by a microsensor-array GC detector is described. The detector comprised an array of four chemiresistors coated with different thiolate-monolayer protected gold nanoparticle (MPN) films, which exhibit transient resistance changes upon reversible vapor sorption. The following vapor pairs, and corresponding array response pattern correlation coefficients ([Rho]), were tested: methyl isobutyl ketone and toluene (MIBK + TOL, [Rho] = 0.81), 1-octene and butyl acetate ([Rho] = 0.59), and nitroethane and cyclohexane ([Rho] = -0.51).

After calibration with individual vapors, binary mixture chromatograms were generated with an upstream separation column for resolution values, R[s]/s, of 0.1, 0.5, and 1.0, and for relative response ratios (RRR) ranging from 1:10 to 10:1. All responses were found to be linearly additive. With proper pre-processing to account for non-uniform peak tailing among the sensors, EFA correctly determined the rank of the composite peaks in 17/21 cases (81%) with R[s]/s = 0.1 and in 39/42 cases (93%) with R[s]/s = 0.5 or 1.0, with most failures confined to the highly correlated MIBK + TOL pair. EFA-ALS recovered response patterns and elution profiles with sufficient accuracy to differentiate the components in 124/126 cases (98%), with 90/124 (73%) quantified to within 30% of actual values. NSF funding.
In the current detector market, the mass spectrometer has been accepted as the most universal detector for gas chromatography with capabilities of both trace quantitative and definitive qualitative information. This being said, quantitative detection can be limited for small or ionizably labile molecules while qualitative shortcomings can be found by some isomeric, isobaric, or structurally similar molecules. The vacuum ultraviolet (VUV) detector we are introducing represents the first fully universal quantitative and qualitative detector option for GC. All species are able to absorb energy in the VUV spectral range (115-180 nm), and their gas-phase spectra are unique across this range. We have demonstrated exceptional sensitivity in the low to mid picogram range for all compounds evaluated, including linear and branched hydrocarbons, polyaromatic hydrocarbons, fatty acids, and pesticides. Spectral deconvolution of compounds indistinguishable by MS that coelute in GC separations is highlighted. Compound classes are able to be identified through systematic absorbance regions in the VUV wavelength range. It is anticipated that the advantages in using this spectroscopic detector can circumvent many of the detection difficulties found with mass spectrometry.

Keywords: GC Detectors, Identification, Pesticides, Petrochemical
Application Code: General Interest
Methodology Code: Gas Chromatography
The presence of even small amounts of elemental sulfur in natural gas is known to seriously affect the distribution lines. Just as dry solid, sulfur will accumulate in valves and fittings, but in the presence of moisture it will be very reactive causing corrosion problems, increasing the costs of maintenance and the risk of safety issues. Now, if the sulfur gets throughout the system and to the final user, the environmental and health problems can be very serious indeed. Just the sulfur fumes can cause irritation of the eyes, nose and respiratory tract. In the presence of O2, elemental sulfur turns easily into SO2 which can cause vascular damage in the brain, heart and kidneys, enzyme system misbalance and so forth. So, it is clear the importance of finding a way to detect and quantify elemental sulfur in the natural gas.

Last year we presented a new approach to fulfill this need. In the present work we want to share new results and advances in our way to develop a method for collecting and analyzing elemental sulfur in natural gas. 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 send to the lab, where is washed with a known volume of a solution of triphenylphosphine and n, n-dimethylformamide in a blend of aromatic solvents, in order to get the sulfur in a stable organic compound: (C6H5)3PS. This solution has been analyzed with a series of different sulfur detectors; a comparison between these results is presented.

Keywords: Gas, Gas Chromatography, Sampling, Sulfur
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography
While the FID is an excellent detector for gas chromatography, there are times where a GC user might wish to use an alternative detector, such as avoiding the use of a flammable gas in a facility. For many of these applications, the argon ionization detector (AID) is an ideal substitute detector. This presentation will provide an overview of the non-radioactive argon ionization detector, discuss where it might be useful to use an AID instead of an FID and provide some exemplary applications such as the measurement of ethanol in beer and wine samples and THC content from medical herbs.
Pesticides are chemical agents used to eliminate unwanted insects from damaging food crops. Although very potent and effective, pesticides can leach into the environment and also are harmful to human beings. Resources like water and various food crops can be easily compromised, due to the widespread use of pesticides. To determine if pesticides are present in samples, a method must be developed to separate these compounds in a systematic fashion from the sample matrix. In the current study, methods were developed for the separation and analysis of pesticides listed in USEPA Method 508.1. Three calibration mixtures and novel Nano Stationary Phase (NSP) GC capillary columns were used. Methods were developed using gas chromatography-mass spectrometry (GC-MS) and gas chromatography electron capture detector (ECD). The analyses of all 17 components in a Calibration Mixture No.1 in 8.60 minutes, Calibration Mixture No. 2 with 9 components in 4.80 minutes, and Calibration Mixture No.3 with 8 components in 4.22 minutes were achieved. A method was also developed for the separation of combined pesticide mixtures No.1, No. 2, No. 3, of the USEPA method 508.1. The 34 component mixture was separated in 14.30 minutes.
With the increasing price of helium it is desirable to conserve helium for applications that require it either by regulatory method or by application. However, in some cases, chromatographs used for these analysis are only operated for a portion of the day. In these cases, in order to conserve helium and still meet regulatory requirements, carrier gas switching can be used for regulatory methods while less expensive and more abundant gases can be used while the instrument is idle. This poster will present an example of this switching mechanism, the necessary operating conditions and the constraints of carrier switching on Hewlett Packard or Agilent gas chromatographs.
Total hydrocarbon analysis is an important measurement in a number of applications including trace hydrocarbon analysis or mud logging. Rather than utilize a separate space consuming and expensive dedicated THC instrument for the analysis of total hydrocarbons, it is possible to use a second channel of a gas chromatograph to acquire this data. In this poster we will present two different versions of total hydrocarbon analysis on gas chromatographs that were also being used for specific component analysis. Set-up and operation of each mode of total hydrocarbon analysis will be presented as well as specific advantages and disadvantages of each operating mode will be discussed.

Keywords: Gas, GC Detectors, Hydrocarbons, Petroleum
Application Code: General Interest
Methodology Code: Gas Chromatography
It is frequently desirable to quantitate very low levels of one component while simultaneously quantitating very high levels of another component. Such an analysis is useful in many industries, including the pharmaceutical industry where both an active ingredient and impurities need to be documented for regulatory agencies. It is also useful in the food industry where an accurate value for both major and minor components need to be determined for proper process control. In this work, two classes of components are introduced by automated headspace analysis and separated on a single GC column. The peaks are then split at a ratio greater than 50:1, with the large fraction going to an Electron Capture Detector (ECD) for ppb level analysis, and the smaller fraction going to a Flame Ionization Detector (FID) for percent level analysis. Calibration curves were generated for both detectors and replicate injections were performed to confirm that the technique is both linear and reproducible.
The use of biogas adds to the diversification of energy supply and reduces emissions of carbon dioxide. Unlike natural gas, biogas produced from fermentation may contain a wide variety of impurities. Operators of natural gas grids and refuelling stations set requirements for the maximum contents of these impurities in biogas to protect the infrastructure and to avoid hazardous situations at the end users. Siloxanes and ammonia are frequently encountered impurities. For siloxanes, methods for preparing calibration gas mixtures and the determination of their contents have been developed. A stability study indicated that these components are not unconditionally stable, but that there is a good potential of providing such mixtures with a relative expanded uncertainty of less than 5% on the fractions of the siloxanes. With respect to ammonia, the feasibility of developing a reference method and measurement standards has been investigated. A reference method with less than 3% relative expanded uncertainty is within reach. A key challenge still remains the provision of reference gas mixtures in cylinders at levels below 30 µmol mol⁻¹ with an acceptable stability period.
Carrier Gas Selection for Capillary GC: There is More Than One Right Answer

Helium is the carrier gas of choice for most US-based gas chromatographers. It has desirable chemical and physical properties, is inert and non-toxic, and has favorable Van Deemter properties. However, it is a non-renewable resource whose cost is rising as its availability decreases. Alternate choices for a carrier gas such as hydrogen and nitrogen are more readily available and can be generated directly in the laboratory. In this report we evaluate some of the practical issues arising from use of these two alternatives to helium as carrier gases.

Hydrogen has many attractive features especially when used at high flow rates because of the relatively flat contribution of the C term. Nitrogen is generally not recommended as a carrier because its van Deemter minimum occurs at a low flow rate, leading to longer analysis times. However, this can be mitigated by using fast temperature programming rates and narrow diameter columns.

We will discuss the practical impact of using various carrier gases over a wide range of linear velocities under both isothermal and temperature programmed conditions. The impact on analysis speed, resolution, and elution temperatures will be considered. The experiments were performed on Agilent 7890 and Agilent 6850 GCs.

This research was funded by Axion Analytical Labs, Inc.

Keywords: Capillary GC, Gas Chromatography, GC, Laboratory
Application Code: High-Throughput Chemical Analysis
Methodology Code: Gas Chromatography
Gas Chromatography
Development of a New Gas Chromatographic Column Set for the Analysis of Blood Alcohol Concentration

Blood alcohol concentration (BAC) analysis is one of the most common analytical tests performed by laboratories involved in forensic and medical testing. Chromatographic separation of the target analytes - as well as possible interfering compounds - is critical for the analysis, especially given that FID is not a selective detection technique. Incomplete chromatographic separation will cause a quantification bias, and could invalidate the results of the test. The new BAC Plus column was designed to mitigate these issues.

The objective of this work was to develop a new set of analytical columns suitable for the analysis of BAC samples. This new set of columns was designed to accommodate either 1-propanol or the newer tert-butanol internal standard, which exhibits coelutions on some BAC columns.

The new set of chromatographic columns resolved all compounds of interest for BAC analyses, two internal standards, as well as common interferences. Baseline resolution between the following compounds was tested: acetaldehyde, methanol, ethanol, isopropanol, acetone, 1-propanol, tert-butanol, acetonitrile, ethyl acetate, and methyl ethyl ketone. In addition, methodology for improving resolution of formaldehyde contamination from ethanol was developed, and sample preparation methods to reduce the effect of formaldehyde contamination were investigated. The optimization of BAC method-specific parameters such as flow rate, liner choice, and injection volume will be presented, as well as methodology to deal effectively with formaldehyde contamination.

The use of hydrogen as a carrier gas was also investigated. The combination of cost-effectiveness, higher efficiency, and higher linear velocity makes it a superior choice over helium for many FID-based tests. It was determined that employing a hydrogen carrier had no effect on results obtained on the new BAC Plus column set.

Keywords: Forensic Chemistry, GC Columns, Headspace, Toxicology
Application Code: Clinical/Toxicology
Methodology Code: Gas Chromatography
Ionic liquids demonstrate unique properties as gas chromatographic stationary phases. By varying the nature of the cation, the anion or the linking groups, it is possible to alter the chromatographic properties of the phase. A wide range of these materials are available commercially. These materials possess both high stability and high polarity and have been shown to be effective in the separation of a range of materials including the high resolution separation of cis and trans isomers fatty acid methyl esters. In this presentation, we evaluate the use of six different commercially available ionic liquid stationary phases for the separation of complex mixtures of aromatic compounds such as those found in petrochemicals and fuels such as reformate, gasoline and high purity aromatics. Selectivities obtained for selected pairs of compounds obtained on these phases will be compared to those obtained on conventional stationary phases such as polyethylene glycol.

Keywords: Capillary GC, Gas Chromatography, GC, GC Columns
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography
A supramolecular Nickel (II) porphyrin complex containing four pyridil-bis(2,2’-bipyridil)chloro ruthenium mezo substituents was submitted to successive voltammetric cycles in high alkaline media to produce a supramolecular matrix with Nickel centers linked by [micro]-oxo bridges, producing a high stable thin film able to act as redox mediator for electrocatalytic oxidation of folic acid (Figure). The characterization of electrode surface material was performed by cyclic voltammetry, UV-Vis spectroscopy, scanning electron microscopy and spectroelectrochemical confocal Raman microscopy. The modified electrode was inserted into a batch injection electrochemical cell used for the rapid and precise quantification of folic acid in pharmaceutical products. The favorable hydrodynamic conditions provided by amperometry-BIA association allowed a very high throughput (theoretical sampling frequency more than 360 injections per hour) with good linear range (1 to 100 [micro]mol L[sup]-1[/sup]) and low limits of detection (7.42 x 10[sup]-7[/sup] mol L[sup]-1[/sup]). The electrochemical method was applied for the quantification of folic acid in different tablet samples. The results were comparable with values indicated by the manufacturer and those found using HPLC as recommended in the Pharmacopoeia. The commercial samples were submitted to a procedure in order to removal lactose of tablets excipients, since carbohydrates act as interfering. This procedure together with the electrochemical method showed to be simple, rapid, efficient and an appropriate alternative for quantifying this compound in real

**Keywords:** Chemically Modified Electrodes, Electrochemistry, High Throughput Chemical Analysis, Pharmacuetic

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

**Methodology Code:** Electrochemistry
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: Characterization, High Throughput Chemical Analysis, Instrumentation
Application Code: High-Throughput Chemical Analysis
Methodology Code: Physical Measurements
High-Throughput Chemical Analysis

**Abstract Title**

Accurate Determination of Moisture Content of Soft Contact Lenses by Near-Infrared (NIR) Spectroscopy

**Abstract Text**

Moisture content determination is a popular application of NIR spectroscopy due to the strong absorption bands of water near 1450 nm and 1940 nm. The delicate properties of soft contact lenses require a minimal-contact method for analysis to avoid damage to the lens itself. NIR spectroscopy provides a quick and damage-free solution. Reference values used for calibration were based on gravimetric analysis. To improve the accuracy water content of contact lenses were determined using a KF titration with Oven sampling. The water content of the lens samples ranged from 24% to 58%. A quantitative model was developed using chemometric software using the water absorption band at 1450 nm. A partial least squares regression model was developed to correlate the spectra profile with embedded moisture percentage. A variety of reflectance and transmission sampling presentations were tested and the accuracy and the precision of the sampling methods were compared. Details of correlation values and the standard error of calibration for each sample presentation are summarized.

**Keywords:** Chemometrics, Near Infrared, Spectroscopy, Water

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

**Methodology Code:** Near Infrared
The increasing demands of customers to provide more reliable analysis of trace impurities in products, requires that the analytical chemist be able to adapt methods to these new matrices. A Varian 450 GC was configured with Pulsed Discharge Helium Ionization Detector (PDHID) for the analysis of atmospheric impurities such as H2, O2, N2, CH4, CO, CO2 and a Thermal Conductivity Detector (TCD) for the analysis of helium, dichlorosilane and trichlorosilane impurities in silicon tetra chloride (SiCl4). This study is important to meet the increasing demands of the semiconductor market for consistent quality in the silicon tetra chloride that is used in that industry. The methods developed and characterized in this study have been found to be acceptable to meet our customer needs for this product quality. All calibration linearity studies are well above 0.995 R2 and method detection limits have been determined and will be presented in this study. With the exception of helium, all impurities have been shown to have similar response factors in inert balance gas and silicon tetra chloride balance gas giving merit to the calibration of the system using standards in either helium or argon balance. This particular study also will report the lower limit of detection (LDL), the coefficient of determination (R2), percent relative standard deviation (%RSD) and calibration range for all the trace impurities measured.
Glancing angle deposition (GLAD) was used to fabricate reproducible porous targets from silica as an integrated platform for ultra-thin layer chromatography (UTLC) separation and solid matrix assisted laser desorption ionization mass spectrometry (SMALDI-MS) detection of small molecules. UTLC is a rapid separation method that allows for multiple detection methods, including mass spectrometry, however conventional separation materials can give problems with volatilization in a mass spectrometer. SMALDI can be combined with UTLC, to provide a powerful detection method. SMALDI offers advantages over organic matrix based MALDI methods. Obtaining a sensitive mass spectrum with MALDI requires careful selection of a matrix and meticulous spot development, and there are significant chemical interferences in the low mass range with MALDI, compared to SMALDI. We have previously proven that cobalt and silicon GLAD films effectively eliminate the need for an organic matrix in the low mass region (MW < 2000 Daltons).

GLAD films were fabricated by electron beam evaporation of purified silicon and cobalt onto silicon wafers. Vertical columns were deposited at 86° angle relative to the substrate normal, with substrate rotation at 1.2 rpm and a deposition rate of 0.6 nm/s. Mass spectra were obtained using an AB Sciex Voyager Elite MALDI TOF instrument.

1 micrometer thick silica GLAD films on silicon substrates were used to separate and detect mixtures of lipophilic dyes, peptides and carbohydrates. For dyes, the RF values ranged from 0.4 to 0.5, for peptides, from 0.3 to 0.8 and for carbohydrates, from 0.3 to 0.7. The distinct bands made for quick verification of compounds by SMALDI-MS on the same device. Our results demonstrate an easy to use, powerful system capable of performing both chromatography and mass spectrometric data analysis.

Keywords: Laser Desorption, Mass Spectrometry, Modified Silica, Thin Layer Chromatography
Application Code: High-Throughput Chemical Analysis
Methodology Code: Mass Spectrometry
Inertness tested GC columns and liners are component parts of a systematic approach in achieving an Inert Flow Path for gas phase analysis. When working with active analytes the little things really do matter. Even very small metal surfaces exposed to the sample in the GC flow path can have a large impact on the peak shape characteristics, detection and recovery of acidic, basic, and sulfur containing analytes. Passivated metal parts in the inlet and in the detector are necessary to achieve consistent and reliable results for the bad actors in a variety of sample sets. For trace analysis of active analytes extreme attention to detail is required which means starting with the most inert flow path possible.

Recent application examples investigated with Inert Flow Path Equipped GCs include drugs of abuse in urine and whole blood, environmental pollutants in waste water and sediments, pesticides in food substances, and gaseous sulfur compounds in in low molecular weight fuels. Results will be shown that highlight the impact an inert flow path has on problem compounds in this diverse set of analytes.

Tips for fine tuning a GC flow path for better inertness and system performance are presented from a practical standpoint. Components that have large and small impact on given analytes are highlighted both in terms of what to look for and how to manage unwanted effects. Guidelines for how and when to do effective maintenance are discussed in the context of the analytes.

Keywords: Environmental, Food Science, Forensic Chemistry, Gas Chromatography/Mass Spectrometry
Application Code: High-Throughput Chemical Analysis
Methodology Code: Gas Chromatography/Mass Spectrometry
The construction of electronically tunable wavelength filters is often complex and requires elaborate optical assemblies and control circuitry. Conventional technologies include liquid crystal-based filters, acousto-optic tunable filters, and electronically tuned etalons. In the work presented here, we describe a novel surface plasmon-coupled tunable filter that incorporates a simple optical design and control circuit. The compact design and rugged optical assembly make it suitable for hand-held hyperspectral imagers. The surface plasmon device requires p-polarized light, but has no intermediate polarizers. The result is a corresponding increase in throughput compared to liquid crystal-based devices that often employ a series of linear polarizers. The theoretical throughput of the surface plasmon-coupled device approaches 100% for the p-polarization state. In addition, the surface plasmon-coupled filter is not susceptible to the unwanted harmonic bands that lead to spurious diffraction in Bragg-based devices. Hence its spurious free spectral range covers a broad region from the blue through near infrared wavelengths. The spectral performance of the device has been characterized and is presented here along with its design and implementation as a wide-field tunable wavelength filter. Wide-field hyperspectral image results from the compact surface plasmon-coupled hyperspectral imaging system are also presented.

Keywords: Imaging, Instrumentation, Spectrometer, Spectroscopy
Application Code: High-Throughput Chemical Analysis
Methodology Code: Surface Analysis/Imaging
Traditionally, HPLC method development has been a time consuming and labor intensive task. The introduction of UHPLC has considerably shortened the evaluation time of individual analyses, however the overall method development process has remained largely inefficient due to the labor intensive aspects of changing columns and mobile phases and limitations of analyst time. Recently, automated UHPLC method development systems have become available that allow a number of columns and mobile phases to be tested without analyst intervention, greatly increasing the efficiency of the method development process. These automated method development systems are compatible with a number of detection technologies that includes mass spectrometry. The capabilities, operation, separation results, and expected time savings of an automated method scouting system will be presented.
Lab-on-a-CD is a high efficiency microanalytical system for medical and biochemical inspection in which microfluids are driven by centrifugal force. Lab-on-a-CD is suitable for integration of micro channels because solution in the micro channel is transported by centrifugal forth. As the whole channel should be rotated at high frequency, the fluidic control is complex due to uneasiness of the packaging of valves. In the present study, we propose a Lab-on-a-CD which could control fluidics using passive nonmechanical valves and a pump such as capillary valve and siphon pump.

Figure 1 shows analysis unit assuming immunoassay,(1, antigen/simple; 2, 4, washing 3, second antibody; 5, substrate 6, detection; 7, waste; 8, absorption measurement; red part, siphon valve; blue part, capillary valves). The microfluidic analysis units were fabricated using typical sote prototyping technique using PDMS. The siphon side wall is treated to hydrophilic by O2 plasma. Solution in 1 to 4 reservoir are transported to a reservoir 6 after opened the capillary valve by the increase of rotating speed. A sequential behavior of solution sending is shown in figure 2. The chemical unit operations for, immunoassay are binding of second antibodies, washing, and reaction of substrate in the reservoir 6. After each steps, these solutions are transported to reservoir 7 from the reservoir 6 by opening of the siphon pump by decreasing of rotating speed. The substrate into the 5 reservoir reacts to antibodies with enzyme after transported to the reservoir 6. After the reaction, the solution is transported reservoir 8 by opening of the capillary valve. The substrate and a stop solution are mixed in the 8 reservoir, and absorption are measured. Thus, the solution control required to ELISA is successfully achieved.

Keywords: Lab-on-a-Chip/Microfluidics
Application Code: High-Throughput Chemical Analysis
Methodology Code: Microfluidics/Lab-on-a-Chip
A method using Pulsed Field Gradient Nuclear Magnetic Resonance for water in oil emulsion droplet size determination has been optimized and compared with optical microscopy. The correlation between the NMR and microscope was good (0.98 and 0.97) in the emulsions with a water content of 20 to 40% for the droplets in the 50 and 97.5 % volume interval. The precision for the determination of the D3,3 (mean diameter of the volume distribution) value by microscope is worse than that of NMR for emulsions with more than 20% water even when three images were used to calculate this parameter.
Quantitative NMR spectroscopy (qNMR) has become an essential tool for the exact content assignment and quantitative determination of impurities. [1] A main property of 1H-qNMR is, that it is a relative primary method, as the signal intensity is directly proportional to the number of protons contributing to the resonance. [2] It is therefore possible to directly compare the signal intensities of an analyte and a reference standard. Thus, the results are directly traceable to an internationally recognized primary reference standard, and therefore traceability to SI units is obtained [3].

This work shows that 1H-qNMR, combined with metrological weighing, can be optimized to obtain results to certify the purity of organic reference materials (expressed as mass fraction) with less than 0.1 % relative expanded uncertainty (k=2). Following well-defined selection criteria, a set of 13 different chemical compounds is evaluated and certified to serve as internal references for 1H-qNMR measurements. The purity of maleic acid is determined by 6 different 1H-qNMR measurement series and all results show full consistency. In 2 more measurement series, four different nuclei are analysed within the same sample against one calibrator. Even with non-optimised signal intensity ratios and varying signal pattern a high consistency was obtained, demonstrating the validity, accuracy and robustness of 1H-qNMR measurement results. All experiments are performed under ISO/IEC 17025 and ISO Guide 34 accreditation.

Keywords: NMR, Reference Material, Trace Analysis
Application Code: Pharmaceutical
Methodology Code: Magnetic Resonance
Bile salts are biomolecules that are produced in the liver and are responsible for the emulsification of dietary fat and fat-soluble vitamins. Despite their importance in biological chemistry, the structure and dynamics of bile salt aggregation is not well understood. Here electrospray ionization-mass spectrometry with an Orbitrap mass analyzer (ESI-MS), [sup]1[/sup]H nuclear magnetic resonance spectroscopy (NMR), and [sup]1[/sup]H-[sup]13[/sup]C heteronuclear single quantum correlation (HSQC) NMR spectroscopy are used to study solutions of the bile salts cholate and deoxycholate. High-resolution negative ion ESI-MS data suggest that cholate can self-aggregate as a dimer, trimer, and tetramer. The presence of a trimer of sufficient stability to be seen in MS analyses is surprising as it is unaccounted for in common models of bile salt aggregation. [sup]1[/sup]H NMR is used to carefully find critical micelle concentrations (CMC) by investigating the effect of increasing the concentration of cholate on a constant concentration of a probe molecule, in this case (S)-(+)1,1′-binaphthyl-2,2′-diyl hydrogenphosphate (S-BNDHP). HSQC NMR spectroscopy is used to probe intermolecular interaction surfaces at the atomic level. HSQC spectra obtained with different concentrations of sodium cholate show which individual atoms are affected by micelle formation. The NMR-based interaction mapping supports the basic anti-parallel arrangement first suggested by Small 55 years ago, but suggests a skewed angle between monomers that compose the dimer.

This work was supported by funding from NSF-RUI Grant #CHE-1153052.
The use of 2-Dimensional (2-D) Nuclear Magnetic Resonance (NMR) techniques allow for correlations within chemical structures not easily detected in simple 1-Dimensional experiments to be seen. The 2-D technique of Heteronuclear Multi Bond Correlation (HMBC) allows the observation of the correlation of hydrogen’s that are 2-3 bonds away from carbon. The original Bruker pulse program on the instrument required a run time of approximately 13 hours and resulted in an uninterpretable spectrum. A common issue with this technique is the signal produced by the hydrogen directly bonded to carbon interferes with the signal intended to be observed. A pulse program was developed to include a Bilinear Rotation (BIRD) filter to remove the directly bonded hydrogen signal. The pulse program was optimized with the coupling constants of ethyl acetoacetate. The new pulse program was able to reduce the run time of the experiment to 2.5 hours. With the new program 6 of the 8 expected peaks were seen. It is believed that the missing peaks are the result of vibrations within the room that the NMR is in.

Keywords: Magnetic Resonance
Application Code: General Interest
Methodology Code: Magnetic Resonance
Due to continuous developments in spectroscopic imaging (SI) technology, it is now possible to obtain lithium intensities from discrete brain regions. Lithium (7Li) continues to be the drug of choice for acute treatment and long-term prevention of mania and the prophylaxis of bipolar disorder. The mechanism by which lithium exerts its beneficial effect is unknown. Because lithium is a centrally acting drug, its concentration in different regions of the brain is important for its function. Proper and consistent lithium concentration in appropriate brain regions is a consequence of good compliance during long-term prophylactic treatment. Full compliance with the prescribed doses is required to realize the benefits from the treatment. The changes in rat brain lithium that are observed due to a missed dose during prophylaxis as obtained by 7Li spectroscopic imaging technique are discussed here.
Purification of large and small molecules often uses preparative HPLC chromatography. Method development for purification uses scale-up techniques to increase capacity, resolution, and throughput. Column size is a factor affecting the overall HPLC purification performance. Selecting the proper length of column for the sample load will have an impact on three main factors laboratories attempt to optimize: injection run time, purity, and yield. Each laboratory will develop methods to optimize one or two main factors, based on the work flow requirements. By changing the column length alone, many methods can be developed to increase capacity, resolution and throughput needs. The data presented in this application will show that a required outcome to increase peak resolution can be achieved by increasing the column length when sample load volume remains the same. When the desired outcome is to increase load capacity, using a longer column can also result in an increase in throughput.

Keywords: Biopharmaceutical, HPLC Columns, Pharmaceutical, Prep Chromatography
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Photo diode array detector is one of the most commonly used detectors in the high-performance liquid chromatography system. Resolution, sensitivity, linearity and stability of the detector have been improved by the sophisticated technology such as newly developed capillary cell, temperature electric control of optical system, and digital signal processing technique. We have developed a peak deconvolution Analysis function to process derivative spectrum chromatogram extracting the derivative spectrum values at the specified wavelength against retention time. The effect of a target component can be canceled by differentiating at the wavelength of the maximum or minimum intensity of the spectrum. This function utilizes the selectivity of derivative spectrum chromatogram to separate unresolved peaks, to detect impurity and to quantitate target peaks on leading or tailing peak eliminating the effect from background data. This feature is applied to several test samples to evaluate the new deconvolution technique. The evaluation results reported in this study provides a new solution which is useful for the peak separation and the impurity detection.

**Abstract Text**

Photo diode array detector is one of the most commonly used detectors in the high-performance liquid chromatography system. Resolution, sensitivity, linearity and stability of the detector have been improved by the sophisticated technology such as newly developed capillary cell, temperature electric control of optical system, and digital signal processing technique. We have developed a peak deconvolution Analysis function to process derivative spectrum chromatogram extracting the derivative spectrum values at the specified wavelength against retention time. The effect of a target component can be canceled by differentiating at the wavelength of the maximum or minimum intensity of the spectrum. This function utilizes the selectivity of derivative spectrum chromatogram to separate unresolved peaks, to detect impurity and to quantitate target peaks on leading or tailing peak eliminating the effect from background data. This feature is applied to several test samples to evaluate the new deconvolution technique. The evaluation results reported in this study provides a new solution which is useful for the peak separation and the impurity detection.
Methods development for reversed-phase liquid chromatographic (RPLC) separations typically requires many time-consuming steps, including manual preparation and pH adjustment of mobile phases, as well as extensive data processing. Also a single detection technique provides insufficient information for missed peaks and co-elutions: Isobaric compounds can be difficult to distinguish with a mass detector alone, while peak identification with UV is not possible for compounds that lack a chromophore. To address some of these challenges, multiple detectors can be used for analysis of a single sample with each detection technique dependent on a different physical or chemical property of the molecule.

In this presentation, we will describe an efficient methods development approach that combines both dual detection and automated software for mobile formulation. This strategy will evaluate a variety of factors including mobile phase pH, organic solvent, temperature, stationary phase and physical parameters for a systematic method development approach. Manipulation of mobile phase pH will be demonstrated through the use of flexible software, while a comprehensive software for simultaneous analysis of both mass and UV spectral data will allow for simplified data processing. The effect of these physical and chemical parameters will be illustrated with both mass and UV spectral data for peak identification. The benefits of peak tracking with combination of mass and UV spectra will be demonstrated for a variety of compounds including natural products and pharmaceuticals.

Keywords: Liquid Chromatography, Liquid Chromatography/Mass Spectroscopy, Method Development, Pharma
Application Code: General Interest
Methodology Code: Liquid Chromatography
Manual preparative purification of both large and small molecules can be time consuming when large fraction volumes are collected from large volume column loading. Gradient separations with closely eluting peaks often demonstrate separation and isolation difficulties if the manual process for each injection is not consistent. Isocratic separations often rely on column volume to provide sufficient separation for purification with adequate recoveries. Using semi-automation with high capacity loading from an injection pump to continuously inject serially onto the column optimizes the manual injection process, eliminating the inconsistencies with manual injection and increasing efficiencies with isocratic gradients. Data and methodologies discussed in this application show examples of optimized profiles of large and small molecule separations, as well as consistent and high purification recoveries as a result of continuous semi-automated system control.
Pharmaceutical: LC and Data Analysis

HPLC Method Development and Validation for USP Norfloxacin Monograph Modernization

Norfloxacin is a synthetic chemotherapeutic antibacterial agent, occasionally used to treat urinary tract infections.

USP Norfloxacin monograph uses a TLC procedure for chromatographic purity and a non-specific titration procedure for assay. To modernize the monograph, a reversed-phase HPLC method was adopted using a Supelcosil LC-ABZ column thermostated at 60°C, a gradient mobile phase of phosphoric acid solution, pH 2.0 and acetonitrile at 1.4 mL/min, and a UV detection at 265 nm, according to European Pharmacopeia (EP) monograph for Related substances A, E, H and K.

The HPLC method was verified for Organic impurities' procedure. The EP system suitability passed as evident from EP norfloxacin for system suitability CRS, EP norfloxacin for peak identification CRS, USP Norfloxacin RS, and four USP candidate samples for Impurities A, E, H, and K. A [i]Resolution solution[/i] and a [i]Standard solution[/i] were proposed for the intended use of the Organic impurities' procedure. Three norfloxacin samples passed the Organic impurities' test.

The EP impurity method was adopted and validated for Assay procedure. All the impurities from three norfloxacin samples were completely resolved from the norfloxacin peak. The main peaks were spectrally pure. The linearity, accuracy, repeatability, and intermediate precision data all met the validation criteria.

This one HPLC method will be used for both the Assay and Organic impurities' procedures in the USP Norfloxacin monograph.

Keywords: Drugs, Liquid Chromatography, Method Development, Pharmaceutical

Application Code: Pharmaceutical

Methodology Code: Liquid Chromatography
Charged Aerosol Detector (CAD) is a relatively new near-universal detection method for HPLC analysis. Previously, only Refractive Index (RI) detectors could be used for universal detection independent of chemical structure; RI detectors are relatively insensitive relying on the difference of refractive index measured between the analyte and eluent. RI has significant limitations in sensitivity for analysis of saccharides as well as gradient method incompatibility. As a result, the frequency of using CAD is increasing in saccharide analysis.

However, one drawback to the use of CAD is due to the high sensitivity. Bleeding of packing materials from the HPLC column are detected as a noise and cause an unstable base line. Generally speaking, bleeding occurs with silica-based columns and are not suitable for use with a CAD detector.

A polymer-based amino hydrophilic interaction chromatography (HILIC) column has been widely used for saccharides analysis with RID, and has demonstrated minimal bleeding in comparison to its silica-based counterpart by CAD. We made full use of the advantage of the polymer-based packing material which is polyvinyl alcohol, and developed a new HILIC column with a different functional group especially for CAD. The new HILIC column was improved in the recovery ratio of saccharides and it has led to the higher sensitivity analysis needed for saccharides.

In the presentation, the newly developed applications for saccharides by the new HILIC column with CAD are introduced.
Rapid Purification of a Diverse Range of Peptides Using Flash Chromatography with ELSD and UV Detection and a New Wide-Pore C18 Media

Newly discovered peptide sequences are providing novel drug development candidates for use in modern medicines. Purification of natural products and synthetic peptides is an essential step in the drug discovery process, and is typically accomplished using preparative chromatography, which can be expensive and time consuming. Flash chromatography is a fast and cost-efficient approach to purify synthetic peptides and other small molecules. Using flash chromatography can quickly increase overall purity of peptides prior to the next amino acid addition or final polishing on preparative HPLC.

This work demonstrates purification and recovery of a diverse range of peptides using an automated Flash Chromatography System with ELSD and UV detection. A new, small particle, wide pore C18 phase shows high loading capacity and high resolution for peptide purification, allowing higher amounts of peptides to be processed in a single injection compared to preparative HPLC. Automated flash chromatography reduces purification time and solvent use compared to FPLC and preparative HPLC.

Biopharmaceutical, Isolation/Purification, Liquid Chromatography, Peptides
Pharmaceutical: LC and Data Analysis

Fast and Efficient Isolation of Botanical Ingredients Using Automated Flash Chromatography

The advent of the Food Safety Modernization Act has imposed tighter regulations on the dietary supplement and natural products industries. The FDA mandates compliance with new cGMP regulations to ensure that manufacturers provide products that contain the correct ingredients, purity, and composition as specified. This stresses the importance of robust testing methods for identification of botanical ingredients to help detect and prevent adulteration. Here we discuss how Automated Flash Column Chromatography (AFCC) can play an important role in the rapid isolation, purification and characterization of compounds. A new “one pass flash” method eliminates time-intensive sample prep work and complements other characterization techniques, such that dietary ingredients are properly identified with minimal time and expense.

Keywords: Identification, Liquid Chromatography, Natural Products
Application Code: Other (Specify)
Methodology Code: Liquid Chromatography
Delivering large quantities of high purity compounds in the shortest possible time is the goal of a purification chemist. Two of the most popular purification techniques are Automated Flash Column Chromatography (AFCC) and Preparative HPLC. Traditionally, AFCC is characterized by the ability to load large amounts of material and short purification times, while Prep HPLC is valued for high resolution separations resulting in very pure products. As a result, AFCC is typically used as a complementary technique whereby the crude sample is enhanced to a higher level of purity before final purification using Prep HPLC. With the recent advances in AFCC instruments and cartridges, the gap between ‘high speed’ flash purification and ‘high efficiency’ Preparative HPLC purification is rapidly shrinking. In many cases AFCC can deliver large quantities of product comparable in purity to Prep HPLC, with significantly less time and expense. In this study, we evaluate the productivity advantages of AFCC over Prep HPLC, in terms of time, solvent and cost savings. We also demonstrate the benefits of AFCC both as a complementary technique to Prep HPLC, as well as a highly versatile stand-alone technique to deliver high purity separations in a cost-effective manner.

Keywords: Isolation/Purification, Liquid Chromatography, Pharmaceutical, Prep Chromatography
Application Code: Drug Discovery
Methodology Code: Liquid Chromatography
In order to enhance the bio-availability and to minimize the variability on in-vivo absorption, a unique formulation was designed by forming a mutiple-layers coating of a O-Acid, a controlling excipient (core) followed by an isolation excipient mixture (middle layer) and PB-1301 (outer layer) to produce pellets. The coated pellets were filled into capsules for clinical studies. During the formulation development work, it is important to monitor the in-vitro release profiles of not only PB-1301 but also O-Acid from the core of the pellets in order to surely establish the correlation between the two release profiles.

A rapid, selective, and simple RP-HPLC method with UV detection was developed to simultaneously determine the concentrations of PB-1301 as well as O-acid during the in-vitro dissolution study for selecting the lead formulation in an efficient manner. O-acid is a relatively less polar component compared to PB-1301. Chromatographic separation was achieved by using an Atlantis T3 4.6*150 mm, 5 um, C18 analytical column. In addition, unlike PB-1301, O-acid does not have an ideal chromophore for UV detection, a dual wavelength detection (210 nm and 317 nm) method was developed to quantitate concentration release profile of O-Acid and PB-1301, respectively.

The method was validated to be precise, accurate, reproducible and rugged for the simultaneous determination of PB-1301 and O-Acid in a single dissolution study. This poster presents method development, validation and results from sample analysis of various capsule formulations.

**Abstract Text**

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**Keywords:** Dissolution, HPLC Detection, Method Development, Pharmaceutical

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Exploring the Selectivity and Performance of a New Extra Selectivity/Extended Stability Cyano Phase for Polar and Non-Polar Analytes in UHPLC/HPLC Method Development

In this work, we explore the unique properties of the novel ACE CN-ES stationary phase which has been designed to maximise selectivity for UHPLC / HPLC method development activities with superb stability, robustness and reproducibility. The principle mechanisms of interaction with most commercially available cyano phases include dipole interactions from the terminal nitrile moiety, low hydrophobic interactions from the typically short alkyl chain spacer and polar interactions from silanols on the silica surface. With the ACE CN-ES an extended ligand spacer has been employed to increase the weighting of the hydrophobic mechanism yet retain the silanophilic and dipole interactions. The extended spacer offers improved phase stability and alternative selectivity whilst retaining multiple modes of interaction. Using a variety of mixtures, the unique selectivity and orthogonality of the ACE CN-ES for polar and non-polar analyte separations is compared and contrasted to hydrophobic phases (eg ACE C18) and also polar / non-polar retention phases such as ACE C18-Amide (polar embedded) and ACE C18-PFP. Finally, separations in 100% aqueous buffer and 100% normal phase conditions with the ACE CN-ES are demonstrated highlighting the versatility of this novel phase.

Keywords: Chromatography, HPLC, Liquid Chromatography, Separation Sciences
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Solid core particle-based stationary phases are becoming increasingly popular in chromatography. Solid core particles offer the analyst many advantages including speed and sensitivity coupled with a low column back pressure. However, many solid core particle columns currently available have a restricted working pH range for isocratic and gradient analysis. The new ACE UltraCore phases use Encapsulated Bonding Technology and offers SuperC18 and SuperPhenylHexyl bonded phases with an extended working eluent pH range of 1.5 to 11.0. This allows the analyst to fully exploit chromatographic selectivity at low, intermediate and high eluent pH. The Encapsulated Bonding Technology also confers a high degree of inertness to the stationary phases giving superb peak shape for acidic, basic and neutral species across the pH range. This poster describes a simple but powerful ACE UltraCore method development platform. Using a range of analytes with SuperC18 and SuperPhenylHexyl bonded phases with low and high pH eluents and MeOH or MeCN it is possible to fully exploit the chromatographic selectivity ‘space’ for rapid method development.

Keywords: Biopharmaceutical, HPLC
Application Code: Pharmaceutical
Methodology Code: Separation Sciences
Mobile phase pH is a powerful selectivity tuner in chromatography. Under alkaline conditions (up to pH 11.0) however, many silica-based stationary phases will exhibit a significant reduction in column lifetime and performance due to dissolution of the silica base material (removing the flexibility of exploring low and high pH eluents from the analyst). In this poster we present the new ACE UltraCore which offers 2.5um and 5um solid core ultra-inert bonded phase particles that deliver superior peak shape for acidic, neutral and basic analytes across a broad eluent pH range of pH 1.5 to 11.0. Solid core particle technology has gained significant interest in chromatography in recent times as the solid core particle architecture offers advantages such as rapid separations, method transferability and lower back pressure. Many solid core phases currently available have sub-optimal characteristics that include a narrow usable eluent pH range for isocratic and gradient analyses and reduced inertness / peak shape particularly for basic analytes. Using novel bonding technology ACE UltraCore offers SuperC18 and SuperPhenylHexyl bonded phases that deliver superior inertness and superb peak shape (and stability) across a wide pH range allowing the analyst to use low, intermediate or high pH eluents to explore selectivity. This poster compares and contrasts inertness data at intermediate eluent pH for a range of solid core columns. Additionally, chromatographic selectivity data at low and high pH for both SuperC18 and SuperPhenylHexyl bonded phases are explored with a variety of analyte mixtures.

Keywords: Chromatography, Liquid Chromatography, Pharmaceutical, Separation Sciences
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
In many cases, HPLC at normal or even slightly elevated pressures with 3-5µm columns can produce very satisfactory results, achieving separation performance up to 100,000 plates/meter. In some cases, however, where very high sample throughput or extra resolution is needed, the option of performing a UHPLC experiment at much higher efficiency is attractive. This poster will describe a new UHPLC column called TitanTM and the instrument design needed to achieve and maintain separation performance that exceeds 250,000 plates/meter. TitanTM columns employ monodisperse 1.9µm porous silica particles modified with C18 and other stationary phases. Because Titan 1.9µm columns have such narrow peaks, a very low dispersion instrument is critical to maintaining peak width for optimum speed and resolution (1). HPLC instruments have pressure and internal volume limitations so they are disqualified for use. Early UHPLC instruments may have adequate pressure rating; however, they show considerable performance loss when coupled with the latest column designs. UHPLC instruments have been continuously improved with lower internal volume and smoother flow transitions. Techniques will be reviewed for determining whether a particular UHPLC instrument is fit for purpose with a column. Examples will be shown with Titan columns using both suitable and unsuitable UHPLC instruments. Several applications will be featured using UHPLC instruments that meet or exceed the minimum performance standards.

Keywords: HPLC, HPLC Columns, Liquid Chromatography, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Lithium is present in pharmaceutical products as therapeutic drug or as residual impurity due to lithium salts used in the process. It is important to monitor the level of lithium in the product to ensure safe usage. Assay for Lithium include various techniques, such as spectrophotometry, flame photometry, ICP OES or ICP-MS, fluorimetry, potentiometry and ion chromatography. The major challenge in these assays is the interference from other alkali ions, such as Na and K which often coexist with Li. In addition, extensive sample preparation including solid-liquid extraction is often needed to eliminate or reduce matrix interference.

Using HPLC connected with a CAD detector, we developed an assay method for analyzing Lithium in pharmaceutical drug products. The method uses reversed-phase mobile phases and a mixed mode column to separate Lithium from the matrix drug and other alkali metals for quantification. The sample can be dissolved in high organic/Water mixture to fully dissolve the drug and extract Lithium. The method has a linearity range of 5ng/mL – 260ng/mL with R² of 0.999, a detection limit of 0.1ng and quantitation limit of 0.3ng, and accuracy is within 98-100% recovery. The method is simple, sensitive and accurate for quantitation of Lithium in drug products with minimal sample treatment.
Separations for pharmaceutical applications can always benefit from improved chromatographic performance. Whether impurity profiling a drug product, identifying a target compound in a complex sample matrix, separating components in a synthetic reaction mixture or finding a new pharmaceutical compound in a discovery setting, the ability to quickly resolve and identify peaks in a mixture is a powerful tool in advancing discovery or development workflows.

New developments in column technology provide chromatographers with an added dimension to increase the peak capacity of their separations. Gains in column efficiency and thereby separation performance can enable chromatographers to resolve and identify desired components more quickly, potentially lessening the need for further method development. Additionally, the increased robustness of newly developed columns enable separations that currently have sufficient resolution to be run at higher flow rates while maintaining the performance of the original separation. Increased flow rates allow significantly faster runtimes and higher sample throughput, resulting in efficient use of instrument time and overall resource cost savings in the lab. In this poster, we demonstrate the performance gains of sub-2-µm, solid-core particle columns for pharmaceutical applications, including impurity profiling, forced degradation, and natural product analysis.

Keywords: High Throughput Chemical Analysis, Liquid Chromatography, Natural Products, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
The demand for high purity peptides is increasing. The application of peptides as promising therapeutic agents against cancer, diabetes, and cardiovascular disease. Both small synthetic peptides and large cellular produced peptides can be difficult to purify to high >98% levels. This is due to the fact that often peptides are structurally similar and differ by only one amino acid. Optimized purification techniques are required to meet these high purity demands in an economical manner. Reversed-phase chromatography, because of its high resolving power, has been the technique of choice for achieving the high level of purity necessary in the pharmaceutical industry. For industrial purification, important consideration and selection of particle size, pore size, and stationary phase in relation to the peptide can optimize purification. We illustrate how a new 150A reversed-phase media is highly effective at purifying peptides with greater loading capacity and improved productivity compared to competitive media. The media has unique selectivity that can reveal peaks masked by other C18 phases and improves resolution of closely related peptides and impurities for higher purity target peptides. The bulk media incorporates bonded phase chemistries identical to those used in analytical and prep columns, thereby assuring economical method development and reliable scale-up for preparative and process purification.

Abstract Text
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Keywords: Biopharmaceutical, Chromatography, Peptides, Prep Chromatography
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
High-Purity Purification Method for Eicosapentaenoic Acid Ethyl Ester (EPA-EE) by a Newly Developed Reversed-Phase Packing Materials

Polyunsaturated fatty acids (PUFA) including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA) are known to have anti-atherogenic effect and anti-hyperlipidimic effect. Recently, market of PUFAs is expanding. Especially, demand for high purity EPA ethyl ester (> 95%) as an active pharmaceutical ingredient is increasing. For purification of those PUFAs, liquid chromatography method is widely used. In order to meet such demand, we have developed a reversed phase packing material designed for highly efficient purification of EPA ethyl ester. By using a combination of this novel chromatography resin and a large scale dynamic axial compression column, we have successfully developed a highly efficient and highly productive method for purification of EPA ethyl ester. In this poster, we will show the actual method development and operation of EPA ethyl ester purification.

Keywords: Liquid Chromatography, Natural Products, Pharmaceutical, Prep Chromatography
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Here, we report a high performance liquid chromatography (HPLC) method to determine the enantiomerization energy barrier of four penta-helicene analogs. The racemization of the helicenes was evaluated dynamically with HPLC using a chiral isopropyl - carbamate cyclofructan 6 (LARIHC CF6-P) column under normal phase conditions. Varying the column temperature and flow rate allowed for the determination of rate constant for the racemization process. A computer assisted deconvolution method was employed to determine the individual peak areas and the retention times for the calculation of enantiomerization energy barrier for these analytes. Apparent enantiomerization energy barriers, enthalpy and entropy for the interconversion of helicenes analogs were determined. These empirically determined numbers were also compared with theoretically determined values.

Keywords: Chiral, HPLC, Liquid Chromatography
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Packaging Selection for Stability Studies and Bulk Storage of Hygroscopic Compounds

The impact of different packaging materials and container sizes on the moisture uptake of hygroscopic APIs was studied. The packaging used in stability programs of hygroscopic APIs should be the same or simulate the packaging proposed for API long term storage. Discrepancies were observed for several programs between analysis of stability samples and bulk API. These discrepancies were mainly due to the small sample size used in stability studies, in comparison to the large sample size for bulk API storage. The goal of this study is to reduce the discrepancies between the two configurations by selecting appropriate packaging for stability studies and API bulk storage.

Hygroscopic compound, PVP K90 (Cross-linked polyvinyl Pyrrolidone), was selected as model compound to study three packaging configurations: One gallon and eight ounce fiberboard drums, and High Density Polyethylene (HDPE) container. Small aliquots of sample were stored in the three packaging configurations in a stability chamber controlled at 30 °C/75% R.H. The moisture uptake profile was evaluated by the weight gain measurements, Karl Fisher titration, and near IR analysis.

A good correlation was achieved among the three techniques. Fiberboard containers of the two sizes showed similar moisture uptake profiles, while HDPE container demonstrated better protection against moisture. The rate of moisture uptake in API stored in full scale fiber-board drums was estimated by measuring the moisture uptake in API stored in small scale drums using the mass balance equations. The resulting analytical expression provides a quantitative model that can be used to predict the mass concentration of water in full size drums based on experimental data obtained from stability samples. Based on sample size and hygroscopicity, an appropriate stability packaging can be selected to simulate the storage configurations for bulk API and to minimize discrepancies between the two configurations.
Scale-up purification from analytical to preparative flow rates and sample loading capacities is traditionally performed by laboratory and process chemists using automated purifications HPLC systems. When run time is a critical factor for laboratory productivity, column diameter versus length is often the parameter selected for scale-up calculations. Traditionally, analytical columns produce good peak symmetry and exhibit high efficiency, while preparative columns can be known for greater tailing and lower efficiency. Changes in the column packing process allow traditional preparative purification to occur with the same column performance, independent of column diameter. This application will illustrate the Suzuki Reaction scale-up of several column diameters, from analytical to preparative, without sacrificing performance for the increase in load capacity. Productivity is maintained by demonstrating consistent peak retention times and run times.
Chiral separation is often required in drug discovery for the screening of natural products, purity analysis of synthetic compounds, and quality control, etc. Cyclodextrins (CDs), especially sulfated CDs, have been widely used as chiral selectors (CSs). Those CSs have become the first choice for quick and dirty chiral separations. We report here the development of a generic approach for obtaining sulfated CDs through simply sulfonation and polymerization process. Thus synthesized novel CSs combine the binding properties of CDs and certain characteristics of polymers, realize the homogenization of substitution degree, and eliminate the disadvantage brought from poor reproducibility. This homogenization reduces the cost of making CSs for CE. The possibility of recycling even further reduces the cost. The factors affecting the sulfopropyl ether [gamma]-CD polymer (SPE-[gamma]-CDP) synthesis process, such as the sequence of reactions, the amount of sulfonating agent, amount of alkali solution in polycondensation, and the amount of the cross-linking agent were investigated here and the resulting products were characterized by elemental analysis, infrared spectroscopy (IR), etc. SPE-[gamma]-CDP, after optimization was synthesized and used for the capillary electrophoretic separation of various enantiomers including neutral, weak acidic, and weak basic analytes with short migration time and high resolution. The different separation conditions, including the concentration of SPE-[gamma]-CDP, the ionic strength and pH of running buffer, separation voltage as well as the addition of organic solution were studied here to get better resolution. Results indicated that the new chiral selector, SPE-[gamma]-CDP, own a unique homogenization of substitution degree and advantages of simple to synthesize, low cost, recyclable, and high enantio-recognition capabilities.
Chromatographic Methodologies Applied in the Purification of Bioactive Molecules in the Venom of Tarantula Spiders

Studies demonstrate that the venom of animals are composed of a complex mixture of small molecules, proteins and peptides. In the venom of the tarantula Vitalius dubius, there is a wide range of peaks can be observed in different methodologies chromatography. Such as reverse phase chromatography, size exclusion chromatography, and ion exchange and bioaffinity. This work demonstrates the use of different methods for the purification of different molecules.
Controlled release of drugs from tablets is a wide-spread method of drug delivery and many formulations use cellulose-based polymers as excipients. These polymers can swell in water, forming a gel layer through which the drug diffuses. Computational modelling of drug release enables more effective formulations to be developed but swelling formulations are challenging due to the moving mass transfer boundaries and complex geometrical changes that occur.

In this work funded by the EPSRC, a novel application of the Discrete Element Method (DEM) is presented, which discretises a swelling tablet into DEM particles and subsequently models drug release. As water diffuses into these DEM particles, a volume change occurs, causing particles to expand and push against their neighbours producing macroscopic swelling and the formation of a gel layer. At the same time, drug within the tablet dissolves and diffuses through the swollen particles, giving rise to drug release.

Optimisation of physical properties and validation of the model was achieved through the use of Fourier Transform Infrared (FTIR) spectroscopic imaging in Attenuated Total Reflection (ATR) mode where tablets containing Hydroxypropyl Methylcellulose and nicotinamide (60% w/w and 10% w/w drug) were imaged as they dissolved, along with downstream UV/Vis to generate drug release curves.

The model was able to extract diffusion properties of nicotinamide from the 60% w/w tablet and predict drug release from the 10% w/w tablet, demonstrating the suitability of DEM for modelling swelling tablets and the powerful combination of FTIR imaging and modelling for formulation optimisation.

Keywords: Imaging, Infrared and Raman, Optimization, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Vibrational Spectroscopy
The content uniformity determination of three actives in Triple FDC tablets has been successfully automated using a TPW (Tablet Processing Workstation) bench-top robotic system. To our knowledge, this is the first TPW method developed for a triple fixed dose combo product. The method was implemented to enable in-process CUDAL testing of drug product process justification batches, which represents a significant number of samples for this fast track project. The data will be used for process robustness evaluation and monitoring plan. During method development, a DoE study with a center composite design was performed to optimize the operating ranges of the critical sample extraction parameters as to determine the interactions between these parameters. The parameters studies were diluent composition, homogenizer speed, time and number of pulses. The detail study design and results will be discussed in this presentation.
The marketing of “pro-hormone” dietary supplements for athletes seeking to increase muscle mass, strength, endurance, and recovery time has increased dramatically in recent years. Stricter drug testing regulations have prompted a few corrupt supplement manufacturers to use chemically-modified structures of existing anabolic steroids, apparently in an attempt to evade detection. These “designer” steroids are expected to convert to active hormones in the body, producing the desired effect. Although little is known about the pharmacological effects of these compounds, it is likely that they, like their banned counterparts, may cause serious long-term adverse health consequences. Several steroid-like compounds in various dietary supplements have recently been detected in our laboratory. These compounds could not be readily identified due to the lack of library reference spectra or commercially available standards. The general analytical approach to these emerging compounds will be presented, including analysis by GC-MS, LC-MS, and/or HPLC-UV. Analytical scale high performance liquid chromatography with fraction collection was used to isolate and collect portions of several new designer steroids observed in samples received. The isolated compounds were then characterized by Nuclear Magnetic Resonance (NMR) spectroscopy and high resolution accurate mass-mass spectrometry (HRAM-MS) to elucidate their structure. Quantitative analysis of these emerging substances in some representative dietary supplements was achieved using HPLC-UV comparison to structurally related reference standards.
There are several important requirements for fast, effective RPLC method development. First, it is important to have columns from a manufacturer whose products have a reputation for quality, reproducibility, and performance. Second, it is prudent to have method objectives clearly defined and documented, and to implement a systematic approach for evaluating and optimizing those important parameters that affect retention, selectivity, and resolution. Finally, it is extremely useful to have a set of complementary stationary phases that have a variety of different retention mechanisms for analyte interactions.

In this poster we will demonstrate the usefulness of a set of new stable, low-bleed stationary phases with unique, complementary selectivities as part of an overall method development scheme. These phases include alkylphenyl, alkylpentafluorophenyl, polar-embedded amide, a broad-pH-range octadecyl, and a unique long-chain alkylcyano phase. An example of such an overall method development strategy using these phases will be presented.

**Keywords:** HPLC Columns, Liquid Chromatography, Method Development

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography
Phospholipids are a broad class of lipids that can be divided into glycerophospholipids (GPLs) and sphingolipids. Both groups show great structural diversity. Phospholipids are amphiphilic molecules, having a hydrophilic head group, and a lipophilic fatty acid tail. Several families of GPLs exist biologically, differing in the type of polar head group present, and include: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidic acid (PA), phosphatidylinositol (PI), dipalmitoylphosphatidylethanolamine (DPPE), dipalmitoylphosphatidylcholine (DPPC), and lysophosphatidylcholine (LPC). Each compound contains many species resulting from differences in their fatty acid composition. For example, PC may contain several different fatty ligands, which will result in multiple peaks by reversed phase chromatography. Differences in polar moieties was used to separate these analytes by normal phase liquid chromatography (NP-HPLC) and provided superior quantitative data with less effort. Analysis of phospholipids in red palm oil and krill oil are provided.

The Corona Veo charged aerosol detector, a sensitive mass-based detector, is ideally suited for the direct measurement of phospholipids, as they are non-volatile and non-chromophoric compounds. It offers excellent sensitivity (down to low nanogram amounts on column), a dynamic range of over 4 orders of magnitude, and similar inter-analyte response independent of chemical structure. The developed method is based on an original publication by Rombaut, R., et al., (J. Dairy Sci., 2005, 88, 482), that enables the direct measurement of a number of GPL and sphingolipid species, each as near-single peaks.

Keywords: HPLC, Lipids, Natural Products, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Abstract Text

Polyphenols are naturally distributed in plants as fruits, seeds, vegetables and tree barks. Some of these compounds are considered beneficial antioxidants for human health. The stem bark of a barbatimão tree, (Stryphnodendron adstringens (Mart.) Coville), grown in the Brazilian savannah has been studied for its traditional use as anti-inflammatory for its contents in proanthocyanidins. The ethanolic extract of S. adstringens stem bark was assessed by 1D reversed phase liquid chromatography (1DLC) with an ultraviolet/diode array detector. Although the organic fraction of previous liquid-liquid purification gave a well defined chromatogram, the crowded chromatographic profile is too complex to be resolved in by a 1D system. Only five compounds including gallic acid, catechin, gallocatechin (GC), epigallocatechin, and epigallocatechin gallate (EGCG) could be identified. GC and EGCG were used for chromatographic quantitative validation in the range 30-330 ng, equivalent to 3-33 µg/ml; r>0.998. The 1DLC method showed good intra- and inter-day precision range (1.72-2.60%) for both markers. Accuracy and coefficient of variation were satisfactory for EGCG (101.8% and 2.32%, respectively) nevertheless, the results were poorer for GC (78.6% and 18.8%, respectively). Due to ease of oxidation of polyphenols, the complexity of the matrices as well as many different driving forces in their separation mechanisms, the 2DLC approach using the UV/DAD or MS detector has been explored since it is a more powerful technique to resolve such samples.

Keywords: Liquid Chromatography, Natural Products, Pharmaceutical, Validation
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
The role of enantioseparation is becoming more and more important especially in pharmaceutical industry. It is known that some enantiomers of racemic drugs show great differences in biological activities such as pharmacology, toxicology, pharmacokinetics, and metabolism. Nowadays, many single-enantiomer drugs are marketed. Although demand for analyses of enantiopurity and enantiopurifications is increasing, there are two difficulties of enantioseparation in the development and production process of chiral drugs; cost of separation and difficulty in method establishment. We have recently developed the chiral stationary phases consisting of polysaccharide derivatives at much lower cost. This greatly contributes to the cost-effectiveness. Additionally, these phases are available in 5 [micro]m material for analytical and 10, 20, 50 [micro]m for preparative. Separation selectivity is identical across all particle sizes. This feature offers predictable scale up from analytical to preparative. In this poster, we will introduce an efficient method development for enantioseparation through some applications. We also show a scaling up example using a semi-prep column and LC-Forte/R preparative HPLC system.

Keywords: Chiral Separations, HPLC, Pharmaceutical, SFC
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Oligonucleotides are short, single-stranded DNA or RNA molecules that have a wide range of applications. The use of oligonucleotides as therapeutic agents is growing annually. Traditionally, reversed phase and anion exchange chromatography have been used to purify oligonucleotides. For large-scale purification, important consideration and selection of particle size, pore size, and stationary phase in relation to the oligonucleotide can optimize purification. We illustrate how a new 150Å C18 reversed-phase media is highly effective at purifying oligonucleotides. The media has unique selectivity that can reveal peaks masked by other C18 phases and improves resolution for higher purity target oligonucleotides. The bulk media incorporates bonded phase chemistries identical to those used in analytical and prep columns, thereby assuring economical method development and reliable scale-up for preparative and process purification.

**Keywords:** Biopharmaceutical, Chromatography, Nucleic Acids, Prep Chromatography

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography
Macrocyclic molecules, such as cyclodextrins and macrocyclic glycopeptides, have been dominant chiral selectors for nearly 30 years. Recently, we have discovered a new member of the macrocyclic chiral selector family called cyclofructan. Cyclofructans are cyclic oligosaccharides consisting of a crown ether core with pendent fructofuranose units spiro-anealated around its exterior. They are formed by the enzymatic conversion of inulin which produces cyclofructans of varying sizes. Cyclofructan 6 possesses an 18-crown-6 and readily complexes with barium. In this study, cyclofructan based stationary phases have been treated with a barium salt solution to create cationic chiral stationary phases. The addition of barium to the chiral selector led to increased retention for sulfonate and phosphate containing chiral analytes in the normal phase and polar organic mode. The increased retention was also enantioselective and chiral separations were obtained using the barium doped columns that were not afforded on the neutral, untreated columns. Namely, the cyclofructan based FRULIC-N, LARIHC CF6-P, and LARIHC CF6-RN. It was determined that all the cyclofructan 6 based chiral selectors bound barium and increased retention for sulfonates and phosphates. Mechanistic and technical aspects of this technique will be further discussed. The developed technique was also applied semi-preparative chiral separations.

Keywords: Chiral Separations, Chromatography, HPLC, HPLC Columns

Application Code: Pharmaceutical

Methodology Code: Separation Sciences
The chromatographic separation of chiral compounds is an important tool in the search for new pharmaceutical entities. Both HPLC and SFC separations of chiral chemicals are important tools for analytical determination and preparative isolation of enantiomeric mixtures. Existing chiral stationary phases can separate a many chiral mixtures. Many of these chiral stationary phases are based on chemically modified carbohydrates. However, even with the existing chemically modified carbohydrates stationary phases there are still many enantiomeric mixtures that are difficult to separate limiting the ability to characterize and purify chemical mixtures containing chiral compounds. In this study we are chemically modifying carbohydrates, such as cellulose, chitin, cyclodextrins and amylose with functional groups that have not been routinely employed. Chemical modifications of the carbohydrates include halogenated, aromatic and hetero-aromatic functional groups. We will present information on the chiral separation characteristics and overall separation capabilities for these chemically modified carbohydrate based chiral stationary phases.
Taste Masking Optimization of an Active Principle Using Taste Assessment by Electronic Tongue Instrument

Many pharmaceutical active principles are known to have a strong bitter taste that can negatively impact palatability. That is why various excipients are used in oral formulations to mask this bitterness. The objective of this study was to investigate the optimal formulation of excipients allowing to achieve the best masking of an active principle bitterness. Four formulations of analgesic syrup containing two different excipients (A and B) combined with two flavouring agents (strawberry and pear) were tested. Taste evaluation was done using the ASTREE Electronic Tongue, a 7-sensor detection system measuring taste compounds dissolved in liquids. Bitterness masking efficiency was evaluated by calculating the taste distance between each formulation and the corresponding placebo consisting of the same composition of ingredients but without the active principle. The shortest the distance, the better the masking. The e-tongue measurement showed that the formulations containing excipient B with either of the two flavors had a low masking power. On the contrary, the formulations containing excipient A proved to be much more efficient in masking the active principle taste. It appeared that the pear flavour was slightly more efficient than strawberry for the taste masking. Using the electronic tongue testing method, a number of candidate formulations could be investigated rapidly. This technique helps significantly improve the taste of oral forms without any safety concerns linked to human testing.

Keywords: Drugs, Integrated Sensor Systems, Pharmaceutical, Sensors
Application Code: Pharmaceutical
Methodology Code: Sensors
A new data processing method for a photo diode array (PDA) detector achieves 10 times wider linear dynamic range. When peak integration of data acquired with the PDA detector exceeds a certain threshold, the method shifts the spectrum to lower UV absorption wavelength automatically, and determines the peak and area from the acquired chromatogram and corrects the absorption ratio between wavelengths using the spectrum information, and then calculates the peak area and height of the target wavelength. It is applied to a pharmaceutical sample Ofloxacin. Following the test procedure in the pharmacopoeia, sample dilution is needed to determine the high concentration component and the low concentration related substances. However, in the dilution process, there is a risk human error occurs unexpectedly. This risk is avoided if all peaks are quantified by a single injection data. The new method is available to record the evidence the sample is properly diluted in the regulation laboratory. In this study, the results are compared between the original method and the new method to show the difference of the major component area values is less than 0.1 %.
### Session Title
Undergraduate Students Only Poster Session

### Abstract Title
Probing Adsorption of Molecular Dyes to ZnO Nanoparticles Using Second Harmonic Generation Spectroscopy

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<tr>
<th>Primary Author</th>
<th>Amani Al-Nossiff</th>
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<td>Co-Author(s)</td>
<td>Chris Nelson, Kevin Shane, Mahamud Subir</td>
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### Abstract Text
Semiconducting nanoparticles, which exhibit electronic and photophysical properties that differ drastically from the bulk crystal properties of the same chemical composition, have found extensive applications in the areas of photocatalysis, optoelectronics, and biosensors. In particular, zinc oxide (ZnO) nanoparticle which has a wide band-gap of 3.3 eV, show excellent promise for the development of dye sensitized solar cells (DSSCs). One of the major obstacles to increase the efficiency of DSSCs is to obtain an optimum condition for the adsorbed dyes. To better understand the mechanism by which dye molecules adsorb to ZnO nanoparticles, we have utilized surface selective second harmonic generation (SHG) spectroscopy. We have found ZnO nanofluid yield strong SHG signal and exploited its intrinsic nonlinear optical properties to understand the binding affinity of various dye molecules. We have also explored the effect of polarization and size of nanoparticles on the adsorption processes.

**Keywords:** Environmental, Nanotechnology, Spectroscopy, Surface Analysis

**Application Code:** Nanotechnology

**Methodology Code:** Molecular Spectroscopy
Undergraduate Students Only Poster Session

Investigation of the DNA Interaction of Novel Photoactive Diimine Complexes of Cr(III) Using LC-MS

Cisplatin and related compounds are well known for their ability to inhibit DNA replication, yet the deleterious side-effects of these Pt(II) complexes encourages investigation for more selective therapies. We are investigating novel Cr(III) complexes with the potential to be used in photodynamic anti-cancer regimens through DNA photocleavage and/or the formation of permanent Cr:DNA adducts. We have worked to characterize the interactions of plasmid DNA, calf-thymus DNA, and synthetic oligomers with newly prepared, heteroleptic [Cr(diimine)3][sup]3+[/sup] complexes containing a range of ancillary diimines and at least one intercalating ligand (e.g., DPPZ, dipyridophenazine). This has resulted in an array of target compounds displaying different DNA affinities (i.e., K[sub]DNA[/sub]), DNA selectivities, and tunable oxidizing potentials. Following irradiation at 350 nm, these complexes may oxidize and potentially cleave DNA owing to their long-lived [sup]2[/sup]E[sub]g[/sub] excited state, with an oxidizing capacity > 1.4 V vs SHE. Further, by substituting monodentate ligands (e.g., 1-methylimidazole) for an ancillary diimine, the formation of DNA adducts is observed by LC-ESI-MS following photoactivation. Data obtained via UPLC and UPLC-MS of custom single-stranded and duplex (hairpin) sequences suggests a pronounced dependency of adduct formation on thymine composition. In an effort to isolate specific Cr(III):DNA adducts, the sequential treatment of samples containing photolyzed Cr(III) and various T primers using enzymatic treatment with BAL-31 (an endo- and exonuclease) and Antarctic Phosphatase (a dephosphorylating agent) is being explored.

Abstract Text

Cisplatin and related compounds are well known for their ability to inhibit DNA replication, yet the deleterious side-effects of these Pt(II) complexes encourages investigation for more selective therapies. We are investigating novel Cr(III) complexes with the potential to be used in photodynamic anti-cancer regimens through DNA photocleavage and/or the formation of permanent Cr:DNA adducts. We have worked to characterize the interactions of plasmid DNA, calf-thymus DNA, and synthetic oligomers with newly prepared, heteroleptic [Cr(diimine)3][sup]3+[/sup] complexes containing a range of ancillary diimines and at least one intercalating ligand (e.g., DPPZ, dipyridophenazine). This has resulted in an array of target compounds displaying different DNA affinities (i.e., K[sub]DNA[/sub]), DNA selectivities, and tunable oxidizing potentials. Following irradiation at 350 nm, these complexes may oxidize and potentially cleave DNA owing to their long-lived [sup]2[/sup]E[sub]g[/sub] excited state, with an oxidizing capacity > 1.4 V vs SHE. Further, by substituting monodentate ligands (e.g., 1-methylimidazole) for an ancillary diimine, the formation of DNA adducts is observed by LC-ESI-MS following photoactivation. Data obtained via UPLC and UPLC-MS of custom single-stranded and duplex (hairpin) sequences suggests a pronounced dependency of adduct formation on thymine composition. In an effort to isolate specific Cr(III):DNA adducts, the sequential treatment of samples containing photolyzed Cr(III) and various T primers using enzymatic treatment with BAL-31 (an endo- and exonuclease) and Antarctic Phosphatase (a dephosphorylating agent) is being explored.

Keywords: Liquid Chromatography, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry, Nucleic Acid

Application Code: Bioanalytical

Methodology Code: Liquid Chromatography/Mass Spectrometry
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<td>Abstract Title</td>
<td>Analysis of Cr(III)–Based DNA Photocleavage Agents Using CGE, PCR and Gel Electrophoresis</td>
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<td>Primary Author</td>
<td>Yasmín R. Alvarez-García, Furman University</td>
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**Abstract Text**

[Cr(diimine)[sub]3[/sub]][sup]3+[/sup] complexes may hold significant promise as potential chemotherapeutics in the field of photodynamic therapy owing to their long excited state lifetimes ([sup]2[/sup]E[sub]g[/sub]) and high oxidizing power. Previous work in our labs using a variety of methods has demonstrated that UV excitation of heteroleptic complexes of Cr(III) containing the intercalating diimine dipyridophenazine (DPPZ) can effect permanent DNA damage via strand cleavage. In the work presented here, gel electrophoresis, capillary gel electrophoresis (CGE, using a recently introduced microcapillary system) and the polymerase chain reaction (PCR) are used to characterize strand cleavage following the incubation of DNA with Cr(III) compounds in the presence of 360 nm radiation. The comparative success of amplification of DNA vectors in the range of 128-144 bp is followed via CGE as a function of irradiation intensity for a range of novel target complexes including [Cr(DPPZ)[sub]3[/sub]][sup]3+[/sup] and [Cr(diimine)(DPPZ)(1-MeImid)[sub]2[/sub]][sup]3+[/sup] where 1-MeImid = 1-methylimidazole. The separation quality, efficiency and amenability to quantitation for agarose electrophoresis versus CGE is discussed.

**Keywords:** Capillary Electrophoresis, Nucleic Acids  
**Application Code:** Bioanalytical  
**Methodology Code:** Capillary Electrophoresis
A diverse group of cationic biocides are used in multipurpose contact lens solutions (MPS) to inhibit bacterial and fungal growth. Maintaining appropriate levels of these agents is critical in preventing infections such as keratitis; thus, sensitive, reproducible, and reliable analytical methods capable of measuring sub-ppm concentrations of biocides are necessary. The most widely used cationic additives are polydisperse (e.g. polyhexamethylene biguanide, PHMB) and/or lack strong UV chromophores (e.g. alexidine dihydrochloride, ADH), complicating their analysis using conventional HPLC with UV detection. Ultra Performance Liquid Chromatography (UPLC) has been investigated for the analysis of PHMB and ADH. Both of these biocides in commercial MPS formulations were separated by reversed-phase UPLC (verified using electrospray ionization quadrupole time-of-flight mass spectrometry) and quantified by their UV response at 220 nm using the method of standard additions with a linear correlation (R\(^2\)) exceeding 0.995. In order to gain insight into the behavior of contact lenses immersed in MPS, uptake studies were performed with ADH and a commercially available daily-wear soft contact lens material. Lenses were immersed in MPS for varying amounts of time of to 5 hours, and chromatographic response was used to calculate remaining biocide concentration. The results clearly demonstrate very substantial uptake of these biocide materials by the contact lens from ADH stock solutions, resulting in a significantly reduced concentration of biocide in small volume contact lens cases.
Gold nanoparticles have been attracting attention due to their unique optical properties, which can be harnessed to increase the efficacy of chemical sensors. Previous studies have established that the swelling or shrinking of a hydrogel in response to its chemical environment can induce an effective change in the refractive index, which, when coupled with the resonant modes of a plasmonic crystal, can be used to detect changes in pH. Further research has revealed that the insertion of gold nanospheres into the hydrogel enhances the sensitivity of the detector, potentially allowing for smaller changes in pH to be monitored. The current focus of our research is understanding how the enhanced electromagnetic fields of nanorods, relative to nanospheres, will engender improved pH sensors. However, integrating water-soluble nanorods into the hydrogel monomer mixture typically leads to aggregation. The detection capabilities of the aggregate are not well defined, which makes them ill-suited for systematically studying sensing processes as a function of aspect ratio. We have determined that a layer-by-layer approach of successive polymer coats stabilizes nanorods embedded in the hydrogel such that these composite thin films can be utilized as sensors.

Keywords:材料特性, 纳米技术, 光谱学, UV-Vis 吸光度/荧光
Application Code: 纳米技术
Methodology Code: 传感器
Nanoparticles have become an increasing field of study due to their optical properties and increased ability of shape selective synthetic methods. As nanoparticles gain traction in fields such as drug delivery and biological sensing, it is important to gain a fundamental understanding of how small molecules interact with the particles. Our work uses gold nanorods that have been synthesized via the seed mediated growth mechanism resulting in a surfactant bilayer of cetyltrimethylammonium bromide (CTAB) on the surface. Two naphthol derivatives, 6-methoxy-2-naphthol and 4-methoxy-1-naphthol, have been used to determine the partitioning coefficient of the hydrophobic molecules into the surfactant bilayer on the surface of the gold nanorods using UV-Vis spectroscopy. By quantifying the partitioning coefficient and number of molecules that partition into the surfactant bilayer, the impacts of the molecular properties, specifically polarity, have been determined. The partitioning coefficients for the two molecules have been determined for a given nanorod aspect ratio, and those results have been compared to a nanosphere. This approach has provided some quantifiable measurements that help to describe the CTAB packing as a function of particle shape, which has engendered increased understanding of the role that CTAB plays in the synthesis.

**Keywords:** Biotechnology, Nanotechnology, Surfactants, UV-VIS Absorbance/Luminescence  
**Application Code:** Nanotechnology  
**Methodology Code:** UV/VIS
Abstract Text

Redox flow batteries are a promising technology for the storage and controllable release of electrical energy through the use of soluble redox chemical species. To be competitive, these redox species must engage in facile electron transfer on inexpensive electrodes and display a high energy density, for which an increased solubility, charge capacity and standard potential must be balanced with their chemical stability and that of the solvent. The purpose of this work is to examine non-aqueous systems in order to expand the functional window of the redox flow battery while increasing its current density and chemical performance.

Here, we focus of on the electrolyte solution to be used in the negative potential portion of the cell. We examine the electrochemical properties of highly concentrated solutions of small organic redox couples such as substituted viologens in propylene carbonate with various lithium salt supporting electrolytes in an inert atmosphere box using Cyclic Voltammetry and Chronoamperometry. The redox couples were first investigated with a glassy carbon macro-electrode and then further investigated with gold, platinum, and carbon microelectrodes. Microelectrode experiments allow us to inspect transport parameters in highly concentrated solutions while providing insight about the number of electrons transferred and diffusion coefficients. The results show that the viologens investigated display a highly reversible two-electron process at moderately negative potentials. The use of viologens is promising because of their reversibility, high solubility in propylene carbonate and their amenability to integrate them into chemical macrostructures that can tackle design challenges in commercial redox flow cells.

Keywords: Electrochemistry, Energy, Microelectrode, Voltammetry
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Electrochemistry
Undergraduate Students Only Poster Session

**Abstract Title:** Wetting C18–Modified Nanoporous Silica Particles with Cyclodextrin

Primary Author: Binbin Lin  
University of Iowa

Co-Author(s): Angie S. Morris, M Lei Geng

**Abstract Text:**
Hydrophobically-modified nanoporous materials have been explored for a wide range of chemical and biomedical applications including drug delivery and chemical separations. Although C18-modified silica particles are effective carriers, the hydrophobicity of the nanopore surface renders the nanopores unwettable by aqueous solutions. In this project, we use cyclodextrin to induce the wetting of the C18-modified nanopores. We demonstrate that the pores are effectively wetted by water in the presence of cyclodextrin. The interaction between cyclodextrin and the organic C18 layer is investigated with an environmental probe pyrene to indicate the polarity of the structure formed by cyclodextrin and C18 chains.

**Keywords:** Cyclodextrin, Fluorescence, Nanotechnology

**Application Code:** Nanotechnology

**Methodology Code:** Fluorescence/Luminescence
Haloacetic acids (HAAs) are a class of disinfection by-products produced during the chlorination of drinking water. The HAAs have potential adverse health effects, and thus regulated by the United States Environmental Protection Agency. Most studies have focused on the formation of HAAs in the drinking water distribution system after chlorination has taken place. However, recent research has shown that monochloroacetic acid (MCAA), dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA) are commonly present at the mg/L level in the bulk sodium hypochlorite solutions (bleach feedstock). These three HAA species are also called HAA3. The presence of HAA3 in the bleach feedstock means the HAA3 are dosed into the distribution system when using bleach feedstock for disinfection. Preliminary research has shown that HAA3 forms rapidly and decomposes slowly in the bleach feedstock solutions. However, the formation mechanisms of MCAA, DCAA and TCAA are unknown. Understanding the fate of HAA3 in the bleach feedstock can be determined by spiking concentrations of each HAA3 species into the bleach feedstock. The concentrations of HAA3 are then monitored using post-column reaction-ion chromatography with nicotinamide fluorescence. The bleach feedstock solution is stored at a constant temperature during each spiking study. The procedures and results of these spiking studies for MCAA, DCAA and TCAA will be presented. In addition, the investigation of a chemical relationship between MCAA, DCAA, and TCAA in the bleach feedstock will be explored and presented.

Keywords: Contamination, Environmental Analysis, Ion Chromatography, Trace Analysis
Application Code: Environmental
Methodology Code: Liquid Chromatography
Disinfection by-products (DBPs) are formed during the chlorination of drinking water. DBPs are regulated by the USEPA due to their adverse health effects, and the two major classes of DBPs are trihalomethanes (THMs) and haloacetic acids (HAAs). The USEPA has many standard methods to analyze DBPs in drinking water which are expensive and require skilled operators. Consequently, most water treatment plants are not able to perform the analysis themselves. As a result, an inexpensive and reliable alternative method is needed for water treatment plants to perform daily, routine DBP monitoring.

The Disinfection By-Product Rapid Response kit (DBP-RR) was designed to inexpensively analyze THMs and HAAs in drinking water. There are some difficulties associated with the DBP-RR due to the manual steps involved, which result in greater error, lengthy sample analysis time.

The DBP-RR kit can be automated with flow injection analysis techniques, such as a peristaltic pump, injection valve and flow through fluorescence detector. A ten port sample injection valve is used to load the THMs and HAAs after they have been separated in the CMS device, reactions occur in a mixing manifold, and products are detected with a fluorimeter. The absence of manual pipetting allows for easy analysis and a reduction in error. Standard addition is attractive because it minimizes the effects of interfering species present in the drinking water. MDL, accuracy, precision and linearity studies will be presented. In addition, the semi-automated standard addition protocol will be compared side-by-side to standard methods in real-world drinking water systems.

Keywords: Automation, Environmental/Water, Water
Application Code: Environmental
Methodology Code: Portable Instruments
**Abstract Title**: Determination of Removal Efficiency of Organic Pollutants by Magnetic Particles Using Surface Selective Laser Spectroscopy

**Primary Author**: Cory A. Diemler  
Ball State University

**Co-Author(s)**: Amani Al-Nossiff, Mahamud Subir

**Abstract Text**
Due to their toxicity, removal of organic contaminants such as phenolic compounds from environmental water is essential. In the recent years removal of aquatic contaminants based on magnetic carrier technique, which involves binding of the pollutant molecules onto the surface of magnetic nanoparticles (MNPs), have been found useful because of the ease with which MNPs can be separated from the solution using magnetic field. However, knowledge of adsorption mechanism, which is an important step of the removal process, of organic molecules to magnetic particle is lacking. Our objective has been to use second harmonic generation (SHG), which is a surface selective laser technique, to obtain adsorption isotherms of an array of selected organic compounds at the surface of MNPs. Our SHG results, in conjunction with the measurements based on traditional UV-Vis spectroscopy, show the applicability of SHG in probing adsorption of pollutants to MNPs dispersed in aqueous solution.

**Source of funding**: Indiana Academy of Science

**Keywords**: Environmental/Water, Laser, Spectroscopy, UV-VIS Absorbance/Luminescence

**Application Code**: Environmental

**Methodology Code**: Surface Analysis/Imaging
Aptamer bioinformatics is an emerging and underexplored field. Traditionally, the final stage of the aptamer selection (SELEX) process is cloning selected nucleic acid into a bacterial expression system followed by conventional sequencing. This approach produces sequence data for 10 to 100 aptamer candidates. Recently, high-throughput sequencing has emerged as an alternative approach, yielding tens or hundreds of millions of sequences and giving a more complete view of the selection process. Bioinformatic approaches are necessary to analyze these large data sets. The simplest data analysis identifies most prevalent sequences in the final round of selection. Enriched sequences are assumed to have survived the SELEX process by binding strongly to target. However, this assumption ignores systematic biases that may affect selection. While oligonucleotide libraries containing random regions are often assumed to be truly random, synthetic bias can produce libraries containing multiple copies of over-represented sequences that may persist throughout selection and appear in the final aptamer candidate pool. PCR amplification and single-stranding may also introduce bias. These biasing effects necessitate that overall sequence enrichment relative to the unselected pool is also considered. Deeper analysis goes beyond primary sequence information to identify clusters of homologous sequences and structures. Such analyses can easily be done in the UNIX terminal, using freely available and open-source bioinformatic tools. We will describe the use of such tools in the evaluation of high-throughput sequencing data from cell-SELEX and CE-SELEX experiments.

Keywords: Bioanalytical, Bioinformatics, Data Mining, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Data Analysis and Manipulation
Human epididymis protein 4 (HE4) is a promising ovarian cancer biomarker with potential to detect the disease in earlier, more treatable stages provided that sensitive and specific assays can be designed. Working toward the development of novel HE4 detection methods, we used capillary electrophoresis-based systematic evolution of ligands by exponential enrichment (CE-SELEX) to select nucleic acid aptamers with affinity for HE4-GST, a form of the biomarker with an attached purification tag. Each step in CE-SELEX—formation of aptamer-target complexes, isolation and collection of complexes from free DNA, PCR amplification of good binders, and regeneration of single-stranded DNA—was optimized to prevent loss and improve yields. Following optimization, a naive library of ssDNA was taken through five CE-SELEX rounds to isolate sequences with micro-molar or better affinity for HE4. Negative selection was performed to remove GST-binding sequences from the pool. High-throughput sequencing was performed on the DNA recovered from each selection round, and the resulting sequence data were analyzed using bioinformatics methods. Sequence enrichment, sequence homology, and pool contamination were investigated in silico. Affinity probe capillary electrophoresis was used to determine the dissociation constants of the selected aptamers. Aptamer candidate affinity was further investigated through competitive and direct ELISA. These selected aptamers are a potential tool in the development of more sensitive ovarian cancer assays and theranostic methods.
Decreasing the amount of carbon dioxide in the atmosphere continues to be a major scientific challenge. According to the EPA, human-related CO2 emissions composed roughly 84% of all US greenhouse gas emissions in 2011. Electrochemical reduction of CO2 to alkanes and alcohols is a promising approach to creating easily stored and transported synthetic liquid fuels from renewable energy sources. To date, potential electro-catalysts for CO2 reduction are industrially unfeasible due to a combination of large overpotentials, poor selectivity, and inadequate stability. Elucidation of the role of the surface on the kinetics of the elementary steps for CO2 reduction will be critical in designing better catalysts. I will report on an ongoing study to explore the activity and selectivity of copper-based bimetallic surfaces. Density Functional Theory (DFT) calculations will be utilized to evaluate the thermodynamics and kinetics of the elementary steps for several possible pathways for CO2 reduction. Surfaces composed of copper deposited on bulk metals such as platinum, gold and silver will be examined. The data obtained from these computations will be compared to values for bulk copper surfaces and used to identify potential new catalysts for CO2 reduction.

**Keywords:** Computers, Electrochemistry, Electrode Surfaces, Energy

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

**Methodology Code:** Computers, Modeling and Simulation
## Session Title
Undergraduate Students Only Poster Session

## Abstract Title
Ambient Ionization Mass Spectrometry for Simultaneous Detection of Organic and Inorganic Components of Gunshot Residue (GSR) and Explosives

### Primary Author
Jennifer Speer  
The University of Tampa

### Co-Author(s)
Brian Sanchez, Hilary Brown, Kenyon Evans-Nguyen

### Date
Monday, March 03, 2014 - Afternoon

### Time
- 

### Room
- Exposition Floor, Back of Aisles 1

## Abstract Text
An analytical method capable of detecting both inorganic and organic components simultaneously, with little sample preparation, is being studied using Desorption Electrospray Ionization (DESI) and Direct Analysis in Real Time (DART) coupled with Laser Ionization-Mass Spectrometry (LIMS). DESI is used to detect organic components and soluble salts. LIMS is useful for detecting insoluble solids, such as the metals observed in gunshot residues or potential radionuclides used in radiological dispersion devices (RDDs). In preliminary experiments, black powder, a common explosive used in IED construction, and 50 ng of CsCl, an analog of a likely radionuclide for an RDD, were added to a polypropylene mesh swab and analyzed using DESI coupled to an ion trap mass spectrometer. The black powder fuel (ascorbic acid) and oxidizer (nitrate and perchlorate), as well as the cesium were detected simultaneously. This demonstrates that both organic and inorganic components of a CsCl-based RDD’s can be simultaneously determined, without sample preparation and using readily fieldable mass spectrometry technologies. In more recent experiments, LIMS has been incorporated into the ionization source and used for analysis of refractory materials. Current research is focused on development of a multi-mode LIMS and DESI/ DART source for simultaneous detection of organic gunshot residues (e.g., diphenylamine) and inorganic lead, barium, and antimony oxides.

### Keywords
Electrospray, Forensic Chemistry, LIMS, Mass Spectrometry

### Application Code
Homeland Security/Forensics

### Methodology Code
Mass Spectrometry
Laser ablation (LA) can facilitate direct analysis of solid samples for mass spectrometry (MS), and is often coupled with an inductively coupled plasma torch (ICP). LA-ICP-MS is now widely used for accurate elemental and isotopic analysis; however, the technique is not fieldable, primarily due to the gas and power requirements of the ICP torch. A mass spectrometer system for elemental and isotopic analysis using technology that is amenable to portable instrumentation is being studied. Solid samples are being ablated with an excimer laser and the resulting particle and ion plume will flow through a microwave plasma torch (MPT) and into an ion trap mass spectrometer. Preliminary data confirms that using laser ablation directly coupled with an ion trap mass spectrometer is a viable technique for detecting metals (e.g., lead, cobalt) and refractory compounds (e.g., strontium titanate). Current efforts are focused on enhancing sensitivity by incorporating a custom MPT, a modification of a design by Bilgic and co-workers\(^1\) which is being fabricated and tested. While we anticipate that LA-MPT-MS will have somewhat reduced sensitivity relative to LA-ICP-MS, MPTs, laser ablation and ion trap mass spectrometers can all been incorporated into portable instruments. This unique LA-MPT-MS instrumentation is promising for rapid elemental and isotopic analysis in the field.


Keywords: Forensic Chemistry, LIMS, Mass Spectrometry, Plasma Emission (ICP/MIP/DCP/etc.)
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
The purpose of this research is to synthesize and compare undecylenic and undecanoic valine, leucine, alanine and glycine based amino acid surfactants. The difference between these two groups of surfactants is that the undecylenic surfactants have an alkene group at the end of the hydrophobic tail while undecanoic surfactants have a saturated hydrophobic tail. Aggregation number and critical micelle concentrations of these surfactants were measured using fluorescence spectroscopy. In addition, 2DNMR techniques were utilized to study the structure and identify the regions of hydrogen bonding among the polar head functional groups. The results indicated several differences between these two classes of surfactants. In addition, effect of pH on physical and chemical properties of these surfactants was investigated. Size of both single and double bond surfactants stayed unchanged in pH range of 6 to 12, however aggregation number and CMC of the surfactants were effected. NMR study indicated that change of pH significantly affect the acidic and the chiral protons. Future research will be focused on investigating mechanism of the chiral separation utilizing these surfactants at different pHs.

Keywords: Chiral
Application Code: Pharmaceutical
Methodology Code: Capillary Electrophoresis
A simple and sensitive colorimetric gold nanoparticle probe for detection of melamine in milk products was developed by the reduction of Au(III) salt with sodium citrate. This method is rather simple that does not involve any surface modification of the nanoparticles or the milk samples used in this study. This cost-effective technique allows for rapid and sensitive on-site detection of milk samples tainted with melamine. This technique is based on the fact that the optical properties of gold nanoparticles depend on distance between particles. Gold nanoparticles are aggregated in a neutral media in the presence of melamine in milk sample; this causes an easily measurable change in the absorption spectrum of the particles which can be monitored with naked eye or UV-Vis spectrometer. Here, the color of gold nanoparticles changed from wine red to blue in the presence of melamine and no color change was observed before melamine was introduced into the milk sample. The observed color change is the result of the coupling of the surface plasmon resonance (SPR) between particles in close proximity. This method is also sensitive and so far, we were able to detect 0.50 ppm of melamine in powder infant milk, which is well below the current safety limit of 2.5 ppm in the US.
Grafting of aryldiazonium ions is a versatile and efficient route to electrode modification. Grafting often takes place in a solution of a diazonium salt or in a reaction mixture containing an aryl amine and a diazotizing reagent, and it has been effected through several means including electrochemical and chemical reduction, as well as UV light and ultrasonic wave exposure [1].

Inspired by a published solvent-free grinding procedure for diazonium ion synthesis [2], we report here a new grafting method that involves grinding electrodes in a water paste of an aryl amine, p-toluenesulfonic acid and sodium nitrite. This method allows for the synthesis of diazonium ions and subsequent surface attachment of aryl groups in a single step. We successfully grafted several groups onto glassy carbon electrodes (4-nitrophenyl, 3-nitrophenyl, 4-hydroxy-3-nitrophenyl and anthraquinone) as indicated by characteristic voltammetry of the attached groups. We likewise pursued modification of copper electrodes.

Surface coverage of 4-nitrophenyl groups on carbon electrodes, calculated by integration of voltammetric peaks, exceeded 2 nmol/cm$^2$—higher than that obtained by grinding in solid diazonium salts, and suggestive of more than a monolayer of attached groups. However, the surfaces are not completely blocking to voltammetry of redox probes such as dopamine and ferricyanide and to further electrochemical reduction of diazonium ions. Using amines with bulkier substituents (2-aminoanthraquinone, 4-amino-2-nitrophenol) resulted in lower surface coverage.

This work was made possible by the Virginia Ellis Franta Fund for Chemistry.


Keywords: Electrochemistry, Electrode Surfaces, Electrodes, Voltammetry
Application Code: Materials Science
Methodology Code: Electrochemistry
Cysteine Dioxygenase regulates cellular levels of cysteine by enzymatically oxidizing cysteine to cysteine sulfinic acid (CSA). Improper activity of cysteine dioxygenase has been linked to a variety of neurological and nutritional disorders. Enzymatic turnover is putatively accompanied by a post-translational modification; a covalent linkage between a tyrosine and cysteine sidechain that is referred to as a Tyr-Cys crosslink. Tyr-Cys might function as a cofactor, as in galactose oxidase, to increase the rate of CSA formation, but that has not been definitively established. Tyr-Cys formation has been measured previously by densitometry of SDS gel images, but the non-linear absorbance of protein stains makes measurement imprecise. We have developed a more precise fluorescence detection assay at pH 10 for Tyr-Cys that can be combined with 1H-NMR that detects the methylene protons’ resonances from CSA, cysteine, and cystine (non-specific product). Different preparations of recombinant cysteine dioxygenase provide altered rates of CSA production, due to exogenous iron ions that produce cystine. Cystine detection is difficult using the typical fluorescence-detected HPLC assays for CSA. Here we simultaneously detect Tyr-Cys, CSA, cysteine, and cystine concentrations at various points during enzymatic turnover. Tyr-Cys formation under these conditions was established (in addition to gel electrophoresis, LC-MALDI, and fluorescence) by 2D NMR rather than relying on X-ray crystallography. Our combined NMR and fluorescence method provides a tool to correlate cysteine dioxygenase activity with formation of the Tyr-Cys post-translational modification.
Determining the Weight Percent of Dye in Peeps

Abstract Text
Spectrophotometry is an essential tool for research as it is an analytical method that is presented in many chemistry courses. This project utilizes the method of standard additions to calculate the weight percent of dye in a Peep™. A Peep™ is a common holiday marshmallow candy. Standard additions is a quantitative method which is used to account for non-analyte material (matrix) present in the sample, in this case the sugar and other compounds mixed with the dye. The dyes present in Peeps™ are synthetic, and experiments have been conducted to determine the relationship between synthetic dyes and hyperactivity in children. This project can be used to compare the mass of dye determined in a Peep™ to the allowed FDA and EFSA acceptable daily intake. The acceptable daily intake of Red #3 dye, the dye present in the pink Peep™, in the United States is 2.5 mg/kg bw/day and Europe is 7 mg/kg bw/day. The weight percent of dye in the yellow Peep™ was determined to be <0.1%. This project can also be adapted for other foods with dyes present. The funding for this project came from Maryville University’s College of Arts and Sciences for the purpose of undergraduate research. Future goals for this project are to develop a robust lab procedure to be used in a quantitative analysis course, and for this procedure to be utilized in spectrometer characterization.

Keywords: Absorption, Food Science, Spectrophotometry, UV-VIS Absorbance/Luminescence
Application Code: Food Science
Methodology Code: UV/VIS
Construction and Characterization of a Micro-Fluorescence Spectrometer

Fluorescence spectrometry is an analytical method that allows for the quantification of an analyte in solutions via emission. This project involves the construction and characterization of a micro-scale spectrofluorometer. The general components of a typical spectrofluorometer are a light source with spectrometer, sample compartment, spectrometer, and computer. However, this spectrofluorometer has a radiation source at 350nm which eliminates the need for one spectrometer. The current components of the microscale spectrofluorometer include an ultraviolet light source, sample compartment, and an ASEQ spectrometer connected to a computer with ASEQ software to collect data. The characterization is being completed using a set of quinine standard solutions. The long term goal is to replace the ASEQ spectrometer with a wavelength specific photodiode that functions at a wavelength of 470 nanometers which eliminates the need for a second spectrometer. The micro-scale spectrofluorometer will be a stand-alone device, supporting data collection and analysis using an iPhone and iPad application. At this point, the LOD is 10ppm quinine using an early prototype of this instrument. Plans include making the control and data collection of the instrument completely wireless and creating a design for the finished instrument based on previous work in the lab.

Keywords: Fluorescence, Instrumentation, Luminescence, Spectroscopy
Application Code: General Interest
Methodology Code: Fluorescence/Luminescence
Undergraduate Students Only Poster Session

The Development and Characterization of a Tactical Light Emission System

Current technology has not yet yielded the development of an effective mechanism of identifying personnel with night vision systems in a cost efficient manner. The development of our tactical light emitting system, allows for positive identification, signaling, and communication at ranges of more than 375 meters, through an infrared signature visible only to night vision systems. The Tactical Light Emission System is a versatile, lightweight (less than 3 oz.), and cost efficient ($25 or less) infrared radiation emitting system, capable of flashing an infra-red strobe visible from a conveniently battery operated apparatus. The emitting strobe is visible only to night vision systems in the near and short wavelength region of the spectrum, with a target wavelength of 0.9-1.2 micrometers. The radiation source also has the capability of modifiable frequencies, allowing for specific flash patterns and sequences that can be customized with basic C computer coding. Bluetooth use allots wireless control through iOS applications, enabling instant alteration of power use and strobe frequency. Each system is comprised of two light emitting devices with three infrared LED’s each, positioned antagonistically on the person, two 3V coin batteries, and a single finger-tip sized microcontroller board.

The TLES allows for safe observation from a distance, personnel accountability, target acquiring, distress signaling, and battlefield communications with limited effort and danger designed specifically for military forces, law enforcement, and emergency first responders. This product has the potential to save lives both in the battlefield and here on the home-front.

Keywords: Infrared and Raman, Near Infrared
Application Code: Other (Specify)
Methodology Code: Near Infrared
The Micro-Vis is a low cost, battery powered and portable Vis-spectrophotometer which uses a tiny micro-controller board that operates entirely by wireless communication via a tablet application. The working size of our spectrophotometer is similar in size to that of the first digit of a thumb or the size of half a lip-balm tube. The Micro-Vis has a relatively minimal cost and software that will control the system will be downloadable. The Micro-Vis covers nearly the entire visible spectrum by using low cost LEDs at specific wavelengths in the visible spectrum along with matching photodiodes for photon detection. With the Micro-Vis spectrometer anyone can access results from a secondary location and upload the results to an online file-sharing account. The data is in a format in which it can be viewed in any spreadsheet program. This low-cost, small scale, wireless communication controlled spectrometer has been characterized using red, blue and yellow dyes in standard solutions to generate calibration curves with R2 values of 0.99 or better in all cases.

**Keywords:** Absorption, Spectrophotometry, UV-VIS Absorbance/Luminescence

**Application Code:** General Interest

**Methodology Code:** UV/VIS
Breast cancer is the most commonly developed form of cancer in women. Mortality due to breast cancer is linked to the stage of the cancer when diagnosed, and an earlier diagnosis is associated with higher survival rates. For this reason it is of high interest to develop a technique to detect the progression of breast cancer so treatments and therapies can be applied earlier. A molecular beacon detection method was developed to quantify certain biomarkers of breast cancer metastasis: prolactin inducible protein (PIP), human epidermal growth factor receptor 2 (HER2), and cytokeratin 19 (CK19). The molecular beacons were able to detect target mRNA in high picomolar range for PIP, and low nanomolar range for HER2 and CK19, and fluorescence intensity change is linearly related to concentration of target mRNA. To increase the limit of detection of the assay a preconcentration technique was developed. Target mRNA hybridized to complementary immobilization probes, then was eluted with the respective molecular beacon in a smaller volume, effectively increasing the concentration. This technique has advantages over other methods of quantifying these biomarkers. The analysis time is much shorter, from minutes compared to multiple hours for an RT-PCR assay, for example. The molecular beacon assay is quantitative and sensitive. The preconcentration method decreases the limit of detection without increasing the likelihood of false positives caused by amplification. This will allow breast cancer metastasis to be detected earlier and provide the information necessary to properly apply treatments to increase survival rates. This research is funded by Research Corporation for Science Advancement, Kalamazoo College, and the Howard Hughes Medical Institute.

Keywords: Bioanalytical, Fluorescence, Nucleic Acids, Spectroscopy
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Detection of primary lesions prior to the formation of late-stage metastatic tumors is crucial to decreasing mortality rate in breast cancer cases. Research herein contains assays based around three mRNA biomarkers including cytokeratin 19 (CK-19), prolactin inducible protein (PIP), and human epidermal receptor 2 (HER2). These biomarkers all are expressed in breast tumor tissue. Fluorescent molecular beacon detection was used to determine presence of biomarker mRNA. Single MB-mRNA assays provide evidence of ability to detect biomarker mRNA with complementary MBs. The fluorescent detection of biomarkers described herein offers several advantages over current detection methods, providing quantitative accuracy, low limits of detection, and specificity that is expected to result in few false positives and negatives. Our multiplex assay has the additional advantages of efficiency and speed, allowing for higher throughput of samples. Ultimately, multiplex MB-mRNA assays could allow enhanced accuracy, precision, efficiency, and a further understanding of disease progression. This research is funded by Research Corporation for Science Advancement, Kalamazoo College, and the Howard Hughes Medical Institute.
Characterizing the Surface Topography of Carboxylic Acid/Alcohol Self-Assembled Monolayers on Gold Electrodes

Abstract Text

The surface structure of Self Assembled Monolayers (SAMs) was probed using electrochemistry. To gain information about the surface topography of the SAMs electron transfer was monitored with potassium ferricyanide as the probing agent. The blocking nature of the film indicates organization of the alkanethiols. The concentration ratio of the SAMs (carboxylic acid to alcohol) was steadily changed and the effects of the concentration ratio were studied using the cyclic voltammograms obtained. A concentration ratio of 100% carboxylic acid to 0% alcohol produced organized SAMs with the best blocking films. The opposite produced SAM structures that are more disorganized and more permeable to the probe molecule.

Three gold electrodes with varying surface roughness (evaporated gold, a flame-annealed gold bead, and a polished gold electrode) were used for self-assembly of the SAMs in order to assess surface roughness influence on SAM formation. The evaporated gold formed SAM surfaces with the best blocking properties, the flame-annealed gold bead had SAMs with the weakest structures since the cyclic voltammograms were reversible. Contrary to expectations, the polished gold electrode produced SAMs with more blocking structures than the flame-annealed gold bead. To understand the reason for the odd behavior of the polished gold electrode, AFM (Atomic Force Microscopy) was performed on all three gold electrodes.

Keywords: Chemically Modified Electrodes, Electrode Surfaces, Voltammetry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
In this study, coordination compounds were formed between the ligand, 2,3-butanedione, and transition metals, copper(II) and cobalt(II). Characterization of the complexes was performed using FT-IR spectroscopy with an ATR attachment. The spectra showed that the carbonyl stretch peaks of the liquid diacetyl shifted approximately 100 cm\(^{-1}\). These frequency shifts suggest coordination between the metal and ligand.
Polyurethanes (PUR) are one of the most versatile substances, contributing to the development of our society through the creation of medical devices and other consumer products. A limitation to characterizing polymers is the ability to fully analyze a mixture of compounds that contain isobaric (same-mass) species.

Methylenedianiline (MDA) is a precursor to methylene diphenyl diisocyanate, which is the major hard block component for PURs. The present study is the second step of an ongoing characterization of the MDA dimers (4,4’-, 2,2’-, and 2,4’-). Our focus is on characterizing multimer (trimer and tetramer) MDA species.

The MDA trimer and tetramer conformations have been analyzed using matrix assisted laser desorption/ionization - time of flight mass spectrometry (MALDI-TOF-MS), electrospray ionization - ion mobility mass spectrometry (ESI-IM-MS), and tandem mass spectrometry (MS/MS). These species were characterized in an assortment of MALDI-TOF-MS matrices (e.g., cyan-4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, and dithranol) and with various charge-producing cations (e.g., Ag+, Li+, and Na+), to probe for potential underlying isomeric conformations. The IM collision cross-section values of the parent ions were calculated, identifying the gas-phase ion structures. The MDA tetramer was found to have at least two isomeric conformations, whereas the trimer molecule was identified to have only one conformation. Molecularly characterizing PURs will advance medical and consumer products.

This research was performed by Tiffany M. Onifer, during a summer internship at Vanderbilt University, funded by the Systems Biology and Bioengineering Undergraduate Research Experience (SyBBURE). The work described above is in the process of being written for publication.

Keywords: Mass Spectrometry, Polymers & Plastics, Separation Sciences, Tandem Mass Spec
Application Code: Polymers and Plastics
Methodology Code: Mass Spectrometry
Biodiesel is an increasingly attractive alternative energy source. It is most commonly available blended with conventional petroleum diesel fuel, ranging from 2 to 20% by volume. In order to fully understand the functionality of this fuel, knowledge of its chemical components is necessary. Biodiesels can be synthesized from a variety of feedstocks, such as soy, canola, tallow, and waste grease, among other plant and animal sources. We have determined that these different feedstocks produce a distinct chromatographic fingerprint when analyzed by gas chromatography-mass spectrometry (GC-MS). Since biodiesel is mostly available in blends, it is important to be able to characterize biodiesel in this form.

Thus, the primary goal of this research was to discriminate the feedstock type for various biodiesel-diesel blends. The FAMEs were separated using GC-MS and a polyethylene glycol column (ZB-WAXplus, 30m x 0.25mm x 0.25[μm]). Peak areas were extracted, normalized, and evaluated using unsupervised chemometric methods such as Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). Using both PCA and HCA, the blended fuels were successfully classified by both feedstock type and concentration. The components in the diesel fuel were not found to play a major role in the clustering. The variables that describe the maximum variation in the data set were determined to be the C16 and C18 FAMEs. We have previously shown that this FAME region most effectively discriminates between pure samples, B100, of biodiesel feedstocks. This demonstrates that biodiesels retain their core chemical properties even in complex mixtures.

Funding was generously provided by the Alumni/Parent Summer Science Research Scholarship Fund and the Robert L. Ardizzone Fund for Junior Faculty excellence, both through the College of the Holy Cross.
Abstract Text
In this study, an inexpensive Raman spectrometer was constructed for future laboratory experiments in undergraduate physical chemistry courses at Seton Hill University. A 10 mW green laser diode was purchased as a light source, along with a notch filter, several lenses, and a polarizer. An existing Pasco diffraction grating coupled with a high sensitivity light sensor was used for detection. This Raman spectrometer is sensitive enough to record Raman spectra for neat organic liquids, efficient resonant Raman scattering species, and aqueous salt solutions. Additionally, by interchanging the green diode with a red diode laser, this system was used to determine the kinetics of particle growth by monitoring Rayleigh scattering.

Keywords: Infrared and Raman, Laser, Raman, Spectroscopy
Application Code: General Interest
Methodology Code: Near Infrared
Organic dyes and pigments used by artists are often subject to degradation and fading with exposure to light. This study seeks to observe the routes and kinetics of degradation in organic dyes derived from the roots of the madder plant (Rubia tinctorum) using ultraviolet-visible spectroscopy. Organic dyes were studied in situ using dilute (approx. 10^{-5}M) solutions of pure dyes in water. The solutions were subjected to different conditions and ultraviolet-visible spectra collected. The effects of bleach and peroxide on the dyes were observed as well as the results of light exposure and the subsequent production of reactive oxygen species in situ. Knowledge of how various conditions impact the degradation process can help art conservators make storage and treatment decisions for works of art. Funded by Seton Hill University.
**Session Title**: Undergraduate Students Only Poster Session

**Abstract Title**: Biodegradable Nanofiber Scaffolds for Bone Tissue Engineering

**Primary Author**: Faiza S. Filfil  
**Author**: St. John Fisher College

**Co-Author(s)**: Patrizia Smith, Stephen Boyes

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**Abstract Text**

Tissue engineering has become a developing field in science and technology and can be applied to numerous clinical situations such as spinal fusions, joint replacements, large bone defects and severe nonunion fractures. There has been a clinical need for bone regeneration with an estimated 600,000 grafting procedures performed annually, making bone the second most transplanted tissue. Existing therapies for bone defects and bone substitutes are autografts and allografts, however, each practice has associated risks for the patient. Tissue engineers have recently approached the lack of an ideal bone graft using nanofiber scaffolds. Functioning as a template for tissue regeneration, scaffolds play a role in cell adhesion, proliferation, differentiation and new tissue formation. Polymers have emerged as a dominant role as scaffolds in tissue engineering. Both natural polymers (such as collagen and gelatin) and synthetic polymers (such as poly(lactic acid) (PLA), and poly(glycolic acid) (PGA)) have been used as materials for preparation of scaffolds. Synthetic polymers are often seen as advantageous to natural polymers because of better control of physical properties, are more uniform and are easy to process, however, lack biofunctionality, have poor mechanical properties, and their hydrophobic nature does not allow interactions with cells.

**Keywords**: Characterization, Infrared and Raman, Medical, Spectroscopy

**Application Code**: Biomedical  
**Methodology Code**: Chemical Methods
Undergraduate Students Only Poster Session

Substituent Effects on the Dipole Moments of (2,3,4)-Aminonicotinic Acid and (2,3,4)-Hydroxybenzyl Alcohol Using the Solvatochromic Method

The solvatochromic method was used to determine the change in the dipole moment upon excitation for the following series of molecules: (2,3,4)-hydroxybenzyl alcohol and (o,m,p)-aminonicotinic acid. Hydroxybenzyl alcohols are pharmacologically related to aspirin, while aminonicotinic acids are used in many hair dyes. Fluorescence and UV-vis spectra were collected in a range of solvents to obtain emission/absorbance maxima. For comparison, optimized structures and dipole moments were calculated using Hyperchem.

Date: Monday, March 03, 2014 - Afternoon
Time: 00:00:00
Room: - Exposition Floor, Back of Aisles 1

Primary Author
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Diane Miller

Abstract Text
The solvatochromic method was used to determine the change in the dipole moment upon excitation for the following series of molecules: (2,3,4)-hydroxybenzyl alcohol and (o,m,p)-aminonicotinic acid. Hydroxybenzyl alcohols are pharmacologically related to aspirin, while aminonicotinic acids are used in many hair dyes. Fluorescence and UV-vis spectra were collected in a range of solvents to obtain emission/absorbance maxima. For comparison, optimized structures and dipole moments were calculated using Hyperchem.

Keywords: Computers, Fluorescence, UV-VIS Absorbance/Luminescence
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
## Abstract Text

Arsenic levels in the woods from bridges on Florida Gulf Coast University campus and soils around them were examined using X-ray florescence (XRF). Prior to 2003, wood was treated with CCA containing chromium, copper, and arsenic to protect from rotting but upon risk assessments the EPA has restricted this. Our research aims to determine how much arsenic is present in the wood bridges and whether or not the arsenic from the wood treatment materials has been leached into the soils around them. Soil samples were taken from the immediate area where the wood bridge samples were collected, and approximately 20 to 40 meters away on either side of the bridge. At an entirely separate area (excluding any bridges), some soils were collected as a control. Results show average arsenic levels of 2000 ppm in wood samples and average concentration of 65ppm in the soil samples that are close to the wood samples. Our findings indicate the soil closest to the bridge contained more amounts of arsenic than the soil samples collected away from the bridge and the control samples. Thus, the wood treatment materials contribute to the arsenic pollution in the surrounding soils. Our results also indicate that some of arsenic in the wood treatment has leached into the environment over time probably from rain run off, negatively impacting our soil and probably the water around the bridge. We will continue to collect data to confirm our findings.

### Keywords:
Analysis, Environmental/Soils, X-ray Fluorescence

### Application Code:
Environmental

### Methodology Code:
X-ray Techniques
Quantifying Naphazoline Hydrochloride and Pheniramine Maleate in Ophthalmic Solution Using HPLC

Naphazoline hydrochloride (NH) and pheniramine maleate (PM) are two active ingredients found in certain allergy relief ophthalmic solutions. NH acts as a vasoconstrictor to reduce redness while PM acts as an antihistamine for allergy relief. A method to quantify these two active ingredients was determined using high performance liquid chromatography (HPLC). The best conditions for quantifying NH and PM standard solutions was determined by trying silica and C18 columns as well as a variety of solvents and flow rates. The optimal parameter for standard analysis and component separation is to use a silica column with a mobile phase consisting of 70:30:0.02:0.03 methanol, water, acetic acid, triethylamine. Currently, commercial allergy relief eye drops are being analyzed with these parameters to determine their concentration of NH and PM. If successful, the methods developed in this project will be utilized as a teaching laboratory experiment and the results could be published.

Keywords: HPLC, Optimization
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Lutetium oxyorthosilicate (LSO) is a commonly used scintillation detector in positron emission tomography (PET), a noninvasive diagnostic technique. The current method to synthesize LSO, known as the Czochralski method, is very energy intensive, expensive, and time consuming. The Czochralski method forms single crystal LSO through combining lutetium oxide and silicon dioxide and melting the mixture at high temperatures. The microwave assisted hydrothermal synthesis of the powder form of LSO is being evaluated as a cheaper, more time and energy efficient method. This synthesis process involves combining lutetium and silicon in specified stoichiometric ratios before the microwave radiation is applied. The structural characteristics of the synthesized samples were analyzed using powder x-ray diffraction (XRD). The purity of the samples are evaluated using the ICDD PDF -4+ Database and Bruker DIFFRACPlus EVA software. A Rivetveld Refinement was applied to give the percentages of the different lattices in the sample by the characterization of the crystalline materials. Once the powder form of LSO is synthesized at a high purity and with similar properties to the crystal form, it will have the potential to be used in PET scans, which would greatly benefit the medical field.

Keywords: Microwave, X-ray Diffraction
Application Code: Biomedical
Methodology Code: X-ray Techniques
Undergraduate Students Only Poster Session

**Abstract Title**: Determination of Biogenic Amines in Local Red Wines as Dansyl Derivatives by High-Performance Liquid Chromatography with Fluorimetric Detection

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**Abstract Text**
Biogenic amines exist in fermented foods in varying amounts due to the bacterial degradation of the corresponding amino acid. Their presence in foods such as cheese, wine, sauerkraut, and aged meat is related to food deterioration. Biogenic amines in high concentrations can lead to headaches, hypertension, nausea, and cardiac palpitations. High concentrations of biogenic amines such as tyramine and histamine are likely candidates for red wine headaches (RWH). Due to the high consumption of alcoholic beverages and possible harmful effects, their concentration levels in foods have been investigated using high-performance liquid chromatography after pre-column dansylation has been applied. The present work reports the efforts to reproduce a published method by coupling an existing HPLC with an existing spectrofluorometer to enable LC-UV and LC-fluorescence detection combinations. The instruments have been successfully coupled together, and fluorescence is detectable. This technique is now being optimized for the analysis of red wine samples from local wineries.

**Keywords**: Chromatography, Fluorescence, HPLC, HPLC Detection  
**Application Code**: Food Science  
**Methodology Code**: Liquid Chromatography
Optical rotation and fluorescence behavior of a rigorously purified phenylalanine ionic liquid, L-Phenylalanine ethyl ester bis(perfluoroethanesulfonylethyl) 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 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 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.

Keywords: Chiral, Fluorescence, GC, Thermal Analysis
Application Code: Pharmaceutical
Methodology Code: Molecular Spectroscopy
Analysis by X-Ray Diffraction Supports Microwave-Assisted Hydrothermal Synthesis of Yttrium Barium Copper Oxide

Products from a hydrothermal synthesis technique for yttrium barium copper oxide (YBCO), a high-temperature superconductor, were analyzed using powder x-ray diffraction (XRD). YBCO was first synthesized using solid state and precipitation techniques. The parameters from those traditional techniques were then used as a basis for developing a microwave-assisted hydrothermal method. Various parameters, such as the length of time in the microwave and temperature, were optimized to attempt to produce YBCO as YBa2Cu3O7. This structure is important as it allows for superconducting properties. When YBCO only has five oxygen atoms per formula unit, it has insulating properties. The change from the orthorhombic structure with seven oxygen atoms per formula unit to the tetragonal structure with five oxygen atoms is key in determining conducting versus insulating properties. XRD was used to assess purity as well as to characterize the structure of YBCO in each sample. The microwave-assisted hydrothermal method allows for better reproducibility of the synthesis and a way to reduce energy consumption in comparison with the traditional approaches.

Keywords: Microwave, X-ray Diffraction
Application Code: Materials Science
Methodology Code: X-ray Techniques
Classification of Feedstock Source in Biodiesel-Diesel Blends

Over the past ten years, biodiesel fuels have had a beneficial impact on the environment and the economy. Biodiesel has the ability to power traditional diesel engines without any need to modify the engine itself, yet it is typically blended with diesel (2 to 20% biodiesel by volume). The fatty acids in various plant oils and animal fats are transesterified to produce the fatty acid methyl esters (FAMEs) that make up biodiesel. The FAME composition in each biodiesel varies with feedstock source and thus greatly influences the overall performance and efficiency. In this research, the FAME content in biodiesel-diesel blends of varying concentration (B2 to B20) and feedstock source (i.e. soybean, canola, animal, etc.) was analyzed using Gas Chromatography and Mass Spectrometry with a polar column chemistry (ZB-WAXplus, 30m x 0.25mm x 0.25μm). Peak areas from these chromatograms were extracted, normalized and evaluated using Principal Component Analysis (PCA). Using PCA, the blended fuels were successfully classified by both feedstock type and concentration. In addition, soy and animal feedstocks were combined to understand characteristics of mixed feedstock blends.

We thank Mr. Jerry Richer of the Alumni/Parents Summer 2013 Research Scholarship Fund for financial support of this research.

Keywords: Biofuels, Chemometrics, Fuels\Energy\Petrochemical, GC-MS
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Chemometrics
Methods of analyzing the quality of water are helpful in determining the amount of trace metals in samples. However, the typical methods tend to be expensive and require tedious sample preparation. Voltammetry is a potential alternative that can be used to determine the concentration of trace metals in samples from reduction and oxidation peaks represented by the measurement of current as the potential energy varies in a voltammogram. In this work linear sweep voltammetry and flame atomic absorption spectroscopy (AA) were used to determine the concentration of iron metal in sample taken from Westminster College’s Lake Brittan. The present work is evaluating if voltammetry can offer equivalent if not better performance compared to flame atomic absorption spectroscopy.

Keywords: Atomic Absorption, Environmental/Water, Voltammetry
Application Code: Validation
Methodology Code: Electrochemistry
Disperse Orange 1 (4-Anilino-4-nitroazobenzene) in cyclohexane was analyzed by flash photolysis and UV-VIS spectroscopy. Due to Disperse Orange 1's push-pull characteristics of the \[ \text{p} \to \text{p} \] electrons coming from the electron donating anilino- group and the electron accepting nitro- group at the \( p \) and \( p^* \) positions, its rate constant from cis to trans isomerization can be analyzed through its change in absorbance. A photolytic flash was applied to solutions of Disperse Orange 1 in cyclohexane with varying temperatures of 20 °, 30 °, 40 °, 50 °, and 60 °C. The change in absorbance and return to the original absorbance was used to determine the rate constant and activation energy of the isomerization. The results provide a new strategy for the analysis of light sensitive dyes controlled by conformation change depending on temperature.

Keywords: Analysis, UV-VIS Absorbance/Luminescence
Application Code: Other (Specify)
Methodology Code: Other (Specify)
The yeast Brettanomyces bruxellensis is known to be responsible for the spoilage of red wine. Through the reactions catalyzed by hydroxycinnamic decarboxylase and vinylphenol reductase, volatile phenols are produced. These volatile phenols are responsible for the off flavors associated with spoiled red wine. In this research, two types of yeast are studied: Brettanomyces bruxellensis and Saccharomyces cerevisiae. The latter is more commonly known as baker’s yeast and lacks the enzymes that are responsible for wine spoilage. To study the production of the vinylphenols, a total of six cultures were grown, three for each type of yeast. The cultures were either uninduced, induced with p-coumaric acid, or with ferulic acid, compounds that are substrates in the vinylphenol production pathway. The whole cell lysate activity for vinylphenol reductase was measured for each culture by a decrease in absorbance at 340 nm, which is the absorbance of the oxidizable cofactor NADPH. After the results of the assay are determined, they will be compared to the results in the paper published by Godoy et al.

**Keywords:** Enzyme Assays, Food Science, Method Development, UV-VIS Absorbance/Luminescence

**Application Code:** Food Science

**Methodology Code:** Other (Specify)
Determination of Manganese by Linear Sweep Voltammetry Using Screen-Printed Electrodes

Voltammetry is a type of electrochemistry used for determining concentrations of a specific analyte in solution. This project uses linear sweep voltammetry with screen-printed electrodes to determine the concentration of manganese in local water samples. Using a voltammeter that monitors the change in potential within each sample will be formatted to a curve that will show the concentration for that sample. These results were compared to the concentrations determined by flame atomic absorption spectroscopy (FAAS) for the local water samples. Electrochemistry is not currently part of the chemistry curriculum at Westminster College but with the help of this experiment, we should see that using a voltammeter and taking measurements of potentials is another analytical method to supplement FAAS determined concentrations of manganese in water samples.

Keywords: Atomic Spectroscopy, Electrochemistry, Electrodes, Voltammetry
Application Code: Environmental
Methodology Code: Electrochemistry
The Biochemistry Laboratory course at Westminster College provides students with biochemical lab skills through a sequential series of weekly experiments. Techniques acquired throughout the course include the cloning, protein expression, and purification of E. coli alkaline phosphatase and continue into protein quantification and characterization.

The goal of this research project is to widen the choice of proteins by writing protocols for studying lactate dehydrogenase, similar to those already used for alkaline phosphatase in the current course. To accomplish this, the expression and purification of S. lucasana lactate dehydrogenase was conducted using methods that include protein expression through E. coli BL21 cells by IPTG induced large scale growth, nickel affinity chromatography, and size exclusion chromatography. A Bradford assay has been conducted for analysis of nickel affinity column protein fractions; approximately 13.5 mg of protein was present for a total of five pooled fractions from a total growth volume of 1 L.

Further data obtained will include quantification of protein from the size exclusion column by Bradford assay in addition to an LDH specific activity assay to determine the kinetics for this recombinant protein. Ultimately, the Biochemistry Laboratory course will include the choice of a variety of protein targets, including lactate dehydrogenase.

**Keywords:** Enzyme Assays, Isolation/Purification, Liquid Chromatography, Protein

**Application Code:** Other (Specify)

**Methodology Code:** Liquid Chromatography
Purification of a protein can be preparative, producing a large quantity of pure protein for commercial products such as enzymes or biopharmaceuticals, such as insulin. Purification can also be analytical for the purpose of research about the identification, quantification, and studies involving the protein’s structure, function, and activity. Acquiring the ability to express and characterize proteins is essential to the career of a biochemist. In order for undergraduate students to acquire these techniques for working with recombinant protein, a published protocol is generally used for specific well-studied proteins, such as alkaline phosphatase. In this project, the purification and expression of glucose-6-phosphate dehydrogenase (G6PD) is being investigated in order to create a protocol for future undergraduate students to use in biochemistry lab. The expression of G6PD requires more complex conditions than a typical E. coli overexpression at 37 [DEGREE]C for six hours with induction of 0.1 mM IPTG. In this project, different methods for the over expression G6PD are being explored to determine the optimal method. Temperature changes, induction with different concentrations of IPTG, and different incubation times are being studied to determine their effect on G6PD expression. Upon identification of the optimal protein expression conditions, the protein will be purified with a variety of chromatography methods and the protein activity will be determined through a dehydrogenase assay. Ultimately, students in a biochemistry laboratory course will be able to purify G6PD with protocols developed in this research project.

Keywords: Characterization, Isolation/Purification, Optimization, Protein
Application Code: Laboratory Management
Methodology Code: Other (Specify)
Undergraduate Students Only Poster Session

GC/MS Comparison of [i]Lavandin Grosso[/i] Oil Obtained by Steam Distillation and SFE

[i]Lavandin Grosso[/i] was harvested by hand at Peaceful Acres Lavender Farm in Martinsburg, OH. Oil was extracted by traditional steam distillation at Miami University Middletown and by supercritical fluid extraction with carbon dioxide at The Ohio State University in Susan Olesik’s laboratory. Extracts were analyzed by GC/MS to compare the oils obtained and to compare them to commercially-available essential oils from France, Spain, and South Africa. No major differences were seen due to the extraction techniques; differences occurred due to the source of lavender. Oil yield will be determined with the next harvest. This work was performed to investigate a possible new agricultural endeavor in Ohio; SFE with carbon dioxide could be used to extract oils from several different crops.

Keywords: Agricultural, Flavor/Essential Oil, Separation Sciences, SFE
Application Code: Agriculture
Methodology Code: Separation Sciences
Nitrite is commonly used in the curing process of meats as a preservative for inhibiting the growth of potentially harmful microorganisms and maintaining a natural pink-red color in cured meats. However, nitrites can produce carcinogenic nitrosamines when meat is overcooked or under acidic conditions, such as within the stomach. In this project, a method based on microchip electrophoresis coupled with electrochemical detection (ME-EC) has been developed to detect the presence of nitrite in bacon samples cured with sodium nitrite and with “no added nitrite except for that naturally occurring.” ME-EC was chosen over the more typical Griess assay due to a great degree of cross-reactivity of the Griess reagent with common interferents present in meat samples. In addition, ME-EC can be made portable and allows for sub-minute analysis times. This method employed the use of a 5 cm simple T PDMS microchip with a 15 µm platinum band electrode aligned end-channel. The separation conditions were optimized to separate nitrite from interferences, such as azide, ascorbic acid, and hydrogen peroxide, as well as iodide, which was used as an internal standard in this study. A LOD of 4.9 μM and a sensitivity of 2.6 mM-1 were obtained for nitrite under optimized conditions. Preliminary results indicate that both cured bacon samples and samples with no added nitrite did contain detectable amounts of nitrite. In the future, this method will be utilized to monitor both nitrite and nitrosamines. Thanks to the University of Kansas Department of Chemistry and MicruX Technologies for support.
As the natural gas industry expands, utilization of hydraulic fracturing to obtain natural gas also increases. The hydraulic fracturing process requires millions of gallons of water per well. Over time, these wells produce waste water (produced water) which contains very high concentrations of barium, strontium, sodium, iron, chlorides, and many other substances. Through a collaboration with ProChemTech International Inc., we are optimizing a treatment process for produced water which recovers sellable products in the process. For example, barium and strontium can be recovered as sulfates from produced water. However, due to their similar chemical properties, they tend to precipitate out of solution together. In order to optimize the separate recovery of barium and strontium from produced water, we use experimental design to study how various factors, such as the concentration of precipitating agent, temperature, and presence of a polymer, affect the precipitation process. Flame atomic absorption spectroscopy, inductively coupled plasma-optical emission spectroscopy, and laser induced breakdown spectroscopy are used for analysis of precipitates and filtrates. The LIBS data is explored using chemometrics, with varying pretreatments, to determine the best model for data analysis.
Whiskey is an alcoholic beverage produced through the distillation of fermented malt grain. Its quality is determined by the raw materials and its overall flavor is dependent on the combination of levels of higher alcohols like ethanol and other constituents produced during the fermentation process. Thus, the amount of alcohol in whiskey has become a very important aspect in its production, consumption and distribution. For these reasons, many techniques for measuring the alcohol content and other components of alcoholic beverages have been developed. The characterization of whiskey has become an important topic in the chemical industry. In past years, various techniques have been developed to ensure the quality of whiskeys to its consumers, however these methods are not easily transferred to a system that could be easily used on-site by the distiller. The present study aimed to develop a methodology to effectively and efficiently characterize whiskey quantitatively and qualitatively using a combined capillary electrophoretic and spectroscopic approach. This presentation discusses the total analytical characterization of a variety of commercially-available whiskeys and the development of a portable, cost-effect device that could be utilized on-site for the spirit analysis.

Keywords: Capillary Electrophoresis, Food Science, Molecular Spectroscopy
Application Code: Food Science
Methodology Code: Capillary Electrophoresis
As the U.S. seeks energy independence, natural gas is becoming an important alternative to oil or coal. The hydraulic fracturing used to acquire natural gas from Pennsylvania’s Marcellus shale poses possible risks to groundwater, thus endangering environmental and human health. Suspected groundwater contamination can be tested for the heavy metals barium and strontium, two signature components of produced frackwater. However, frackwater is a complex matrix with high concentrations of salts and metals and it’s very difficult to accurately determine the amount Ba and Sr without encountering instrumental interference. Calcium interferes with Ba analysis using FAAS because it has absorption bands with very similar wavelengths. Both Ba and Sr were isolated from Ca using a Dowex 1x8 (100-200 mesh) ion exchange resin using a nitric acid-methanol mixed solvent. To avoid spectral interference from Ca with flame atomic spectroscopy, Ba was analyzed with an ionic resonance line (455.5 nm) in absorbance mode while using a nitrous oxide-acetylene flame. Strontium was analyzed with Ln and K to suppress ionization at 460.7 nm in absorbance mode. Along with laboratory research, we have also worked with Lawrence and Mercer counties’ citizens by monitoring stream health through conductivity and total dissolved solids measurements. To enhance collaboration of volunteers, a website was created for the Lawrence and Mercer County Alliance for Aquatic Resource Monitoring (ALLARM) program (based on the Dickinson College ALLARM model). The website, which can be found at https://sites.google.com/site/lawrencecountyallarm/, provides free public access to the measurements taken, information on the hydraulic fracturing process, and a map of all the current shale gas wells in the area. Facing possible hydrofracking impacts on their streams, Lawrence and Mercer county communities benefit from the community-based research provided in the form of heavy metal testing and website development.

Keywords: Atomic Spectroscopy, Environmental/Water, Ion Exchange, Metals
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Carbon nanomaterials vary drastically in size, physical characteristics, and applications. In the past years these nanomaterials have received increased attention due in part to their unique physical and chemical properties. Prior to much of the more recent research, carbon nanomaterials were used to encapsulate and amplify the properties of other materials. Now, more research is focusing on the characteristics of the carbonaceous species themselves. Many carbonaceous nanomaterials exhibit characteristics such as semiconductivity and photoluminescence. Photoluminescence is of particular interest in the scientific community due the wide variety of potential applications these nanoparticles hold in the fields of bioimaging, photocatalysis, optoelectronics, and synthetic chemistry. There are many ways to synthesize these carbon nanoparticles, however many methods are costly or require complicated techniques. A straightforward synthesis involving acetic acid and phosphorus pentoxide provides a simple mechanism under which nanoparticles can be synthesized in a reproducible manner. Capillary electrophoresis (CE) provides a way in which carbon nanoparticles can be separated and characterized based on their individual size and charge. The use of CE to generate electrophoretic profiles of the nanoparticles produced with variable post-synthetic treatments can provide for a fast and comprehensive screening tool that can help to identify treatments that select for materials with desired chemical and physical properties. This presentation highlights the use of CE as a characterization tool with an emphasis on the heterogeneity of the nanoparticle sample.

Keywords: Capillary Electrophoresis, Materials Characterization, Nanotechnology, Separation Sciences
Application Code: Nanotechnology
Methodology Code: Capillary Electrophoresis
Continuous monitoring of surface ground water is an essential aspect of environmental analysis. As development of green space becomes more prevalent, it becomes more important for the ability to sense changes in aquatic chemistry at low levels as well as to pinpoint the timeframe from which pollution occurs. One particular source of surface water contamination is the various hydrocarbons associated with petroleum distillates. Periodic, discrete pollution events may release contaminants into the aquatic system that may not be revealed during routine water analysis, however, these events may be detected through the analysis of organisms capable of bioaccumulation. Depending on their lifecycle, a variety of benthic macroinvertebrates have the potential to accumulate a variety of petroleum-based hydrocarbons. This presentation highlights the systematic investigation of the bioaccumulation of petroleum hydrocarbons by a number of benthic macroinvertebrates using solid-phase extraction and gas chromatography/mass spectrometry. Species selection and pollutant rate of uptake will be discussed within the context of organisms harvested from waterways found in the New York’s Southern Tier and Pennslyvania’s Northern Tier regions.

Keywords: Biological Samples, Environmental/Water, Gas Chromatography/Mass Spectrometry, Solid Phase Extr
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
In recent years, our lab has been investigating the interaction between novel tris-diimine Cr(III) complexes and DNA in the development of potential photoactivated chemotherapeutics. These complexes have the ability to photooxidize guanine when excited by UV light, leading to DNA strand scission and, in some cases, permanent adduct formation. By investigating systems that contain an intercalating diimine ligand (e.g., dipyridophenazine, DPPZ; 11,12-dimethyldipyridophenazine, Me2DPPZ) in combination with a range of “ancillary” ligands (e.g., 1,10-phenanthroline, phen; 4,7-diphenyl-1,10-phenanthroline, DIP; 3,4,7,8-tetramethylphenanthroline, TMP; 1-methylimidazole, 1-MeImid), we are able to simultaneously manipulate binding strength, complex enantioselectivity and oxidative power. The current study presents the results of viscometric titrations, which have been used to verify intercalation of several new Cr(III) targets with calf thymus B-DNA. In addition, due to the reduced absorptivity of intercalated Cr(III) complexes, associated with pi stacking with DNA bases, spectrophotometric titration was utilized to quantify binding affinity (K_{DNA}) using the McGhee-Von Hipple algorithm for non-cooperative binding. Isothermal titration calorimetry (ITC) data was also employed to ascertain the contributions of enthalpy and entropy to the thermodynamics of binding, as well as to verify the K_{DNA} values obtained by spectrophotometric titration. Equilibrium dialysis and circular dichroism (CD) demonstrate unique differences in the binding affinities of the [lambda] and [delta] Cr(III) enantiomers as a function of ancillary ligand identity.
BPEs are electrically conductive materials that promote electrochemical reactions at its extremities (poles) even in the absence of a direct ohmic contact. More specifically, when sufficient voltage is applied to an electrolyte solution in which a BPE is immersed, the potential difference between the BPE and the solution drives oxidation and reduction reactions. Because no direct electrical connection is required to activate redox reactions, large arrays of electrodes can be controlled with just a single DC power supply or even a battery. The wireless aspect of BPEs also makes it possible to electrosynthesize and screen novel materials for a wide variety of applications. In this talk we discuss fundamentals and applications of bipolar electrodes (BPEs). A new approach to seawater desalination using BPEs will be a primary focus.

Keywords: Electrode Surfaces, Environmental, Environmental/Water, Separation Sciences
Application Code: Environmental
Methodology Code: Electrochemistry
Detection of Short-Lived Electrode Reaction Intermediates with the Scanning Electrochemical Microscope – Sn(+3) and Others

Scanning electrochemical microscopy (SECM) has made it possible to detect short-lived intermediates of electrode reactions. Because measurements are made at steady state, the difficulties from double layer charging and adsorbed species, that prevail in transient techniques like fast scan cyclic voltammetry, are not a factor. This is useful in diagnosing the mechanisms of multi electron transfer reactions, for example the reduction of Sn(+4) to Sn(+2).

Keywords: Electrochemistry
Application Code: Nanotechnology
Methodology Code: Electrochemistry
Pittsburgh Analytical Chemistry Award

Autonomous Bio/chemical Analytical Microsystems for Space Science: Development of the O/OREOS Nanosatellite and Results from Orbit

Small autonomous satellites under 10 kg, known as nanosatellites or “cubesats”, can reduce the cost and increase the frequency of space science experiments. Exploiting recent advances in nano-, micro-, and miniature technologies in fields from biotechnology to materials to sensors to telecommunications, cube/nanosatellites are under development by over 100 universities, many small commercial entities, major aerospace companies, more than a dozen developing nations, and major space agencies around the world. These small spacecraft can support sophisticated science; the coupling of autonomy and telemetry provides near-real-time kinetic data from space science experiments.

We designed, developed, constructed, tested, and launched, and now continue to analyze data telemetered from low Earth orbit from, the Organism/ Organic Exposure to Orbital Stresses (O/OREOS) nanosatellite. Measuring ~10 x 10 x 34 cm and weighing 5.5 kg, O/OREOS launched late in 2010 into 650-km Earth orbit. O/OREOS comprises three conjoined 10-cm-cubes, each a fully integrated microsystem: a “bus” including communications, power, and control functions, and two science payloads.

The Space Environment Survivability of Living Organisms (SESLO) payload comprises three “bioblock” microfluidic modules, each with 12 75-µL sample wells for growth and 3-color absorbance-based characterization of microorganisms. Soon after orbital deployment and again after 3 and 6 months in space, SESLO recorded the growth and metabolic activity of Bacillus subtilis bacteria.

The Space Environment Viability of Organics (SEVO) payload tracks the photostability of organic molecules relevant to the development and presence of life. Comprising a miniaturized UV-visible spectrometer and 24-sample carousel holding hermetically sealed microcells, SEVO uses the Sun as its light source. The (photo)chemical changes of organic thin films resulting from over one year of full-spectrum solar exposure in orbit will be reported.

Keywords: Biological Samples, Lab-on-a-Chip/Microfluidics, Portable Instruments, Spectroscopy
Application Code: Bioanalytical
Methodology Code: Portable Instruments
Density is an interesting property: all matter has it, and it is thus potentially interesting as a target for new analytical methods. Although there are many circumstances in which it is not possible to measure density, the development of new methods for doing so, and new applications once having done so, have been sparse: it is not a popular target. This talk will outline two new methods of measuring density. One--magnetic levitation--has (depending on how it is used) great simplicity and remarkable sensitivity and/or range. The other--based on MUPS, or multiphase (aqueous) polymer systems--is particularly promising for applications in biology.
Microfabrication and printing techniques have revolutionized the world; however, most are not simple, cheap, and rapid enough to allow for the creation of prototype devices on the benchtop. Recently, massively parallel scanning-probe based methods have been used to address such challenges and mark a step towards the realization of a “desktop fab.” Such a tool should enable simple, flexible, high-throughput, and low-cost nano- and microscale patterning and allow chemists, biologists, and engineers to rapidly synthesize and study systems pertaining to nanoparticle catalysis, single particle electronic devices, and biochemical processes at the cell surface. Specifically, we have been pursuing cantilever-free scanning probe lithographic methods to achieve this goal. These methods are promising in that they are intrinsically low cost, high resolution, and massively parallelizable. Initially, we developed polymer pen lithography (PPL), which uses arrays of elastomeric tips on a rigid backing layer to transfer chemically reactive materials (e.g. alkanethiols, proteins, polymers) in a direct-write manner onto a variety of surfaces. The science and development of PPL has enabled researchers to systematically investigate phenomena in chemistry, biology, and materials science. For example, we have utilized PPL to study the differentiation of mesenchymal stem cells in response to surface patterns of extracellular matrix proteins. In addition to PPL, we have also developed hard-tip, soft-spring lithography which utilizes Si tips to produce sub-50 nm features in a force-independent manner. This architecture is also useful for directing light for both near- and far-field photolithography, a procedure termed beam pen lithography (BPL). Importantly, we have shown that BPL can be massively multiplexed through the use of a digital micromirror device in order to allow one to rapidly produce arrays of arbitrary nanoscale features across centimeter-scale surfaces.

Keywords: Materials Science, Nanotechnology
Application Code: Materials Science
Methodology Code: Other (Specify)
Far-ultraviolet (FUV) spectroscopy is concerned with the 140–200 nm region. This region is very rich in information about the electronic states and structure of a molecule but the absorptivity is very high there. Therefore, FUV region has been employed to investigate mainly for the electronic states and structure of gas molecules. To observe spectra of solid samples in the FUV region, reflection spectroscopy has been used. However, for liquid samples, in general, it is very difficult to use either absorption spectroscopy or reflection spectroscopy. Accordingly, FUV spectroscopy for liquid samples has almost been an undeveloped research area.

To solve these difficulties in FUV spectroscopy we have recently developed a totally new FUV spectrometer based on the attenuated total reflection (ATR) technique that enables us to measure spectra of liquid and solid samples in the 140–200 nm region. This spectrometer has opened up a new era of FUV spectroscopy. My talk consists of seven parts: (i) introduction to FUV spectroscopy, (ii) characteristics and advantages of FUV spectroscopy for the study of liquids and solids, (iii) development of new FUV spectrometers, (iv) FUV studies of liquid water and aqueous solutions, (v) FUV spectra of organic molecules in the liquid states, (vi) potential applications of FUV spectroscopy in liquid and solid states, and (vii) time-resolved (TR) FUV spectroscopy. I will show that FUV holds considerable promise not only in basic science but also in applications such as qualitative and quantitative analyses, on-line monitoring, environmental geochemical analysis, and surface analysis.

References

Keywords: Instrumentation, Molecular Spectroscopy, Spectroscopy, UV-VIS Absorbance/Luminescence
Application Code: Other (Specify)
Methodology Code: Molecular Spectroscopy
Fourier-Transform mid-infrared (FT-IR) and near-infrared (FT-NIR) imaging have over the last decade proved powerful tools for a broad range of industrial and research applications. Despite the awareness of their impact on material science, attempts to further enhance their potential by the use of polarized radiation or by combination with variable-temperature measurements have been rather scarce.

These instrumental issues will be addressed in the present communication, which on the one hand reports imaging investigations of anisotropic materials and on the other hand demonstrates the use of a novel variable-temperature cell to monitor melting and diffusion processes in different polymers by FT-IR and FT-NIR imaging measurements.

Thus, FT-IR dichroic imaging measurements of polymer films which have been previously subjected to mechanical elongation yield detailed information on the lateral distribution of anisotropy. In the case of heterogeneous deformation mechanisms such measurements not only provide a detailed picture of the chain orientation but can also be evaluated in terms of the resulting film-thickness heterogeneity.

Furthermore, FT-IR and FT-NIR spectroscopic imaging measurements with a novel sheet-structured, temperature-controlled sample holder are reported. One application describes the phase separation of a biopolymer blend film as a function of temperature between 25°C and 175°C. In the second example the diffusion process of a deuterated alcohol into an aliphatic polyamide was monitored at two different temperatures in order to demonstrate the significant differences of diffusion rate below and above the glass transition temperature. Apart from the in-situ visualization of the diffusion front in the time-resolved FT-NIR images the type of diffusion and the diffusion coefficient of the investigated process have been determined.

Keywords: FTIR, Imaging, Near Infrared, Polymers & Plastics
Application Code: Polymers and Plastics
Methodology Code: Vibrational Spectroscopy
Vibrational circular dichroism (VCD) has emerged in recent years as a powerful technique for molecular structure analysis in chiral molecules[1]. Most applications have been carried out for natural products and organic molecules of pharmaceutical significance[2] and for molecules of biological significance, such as peptides, proteins, carbohydrates and nucleic acids. Several years ago, it was discovered that protein amyloid fibrils, implicated in many neurodegenerative diseases such as Alzheimer’s disease, show unusually enhanced VCD intensities owing to the long-range supramolecular chiral structure of fibrils[3]. Since then we have developed several lines of research to reveal details regarding the origin, development, incubating pH dependence, morphology and sense of chirality for a variety of amyloid fibrils[4]. Of particular interest are variations in the spatial heterogeneity of fibril films and fibrils in tissues. To this end we have constructed a VCD microsampling accessory to provide measurements of VCD spectra with spatial resolution at the level of 1 mm and below. Recent progress in the VCD microsampling in fibrils samples will be described.


Keywords: Bioanalytical, FTIR, Microspectroscopy, Protein
Application Code: Bioanalytical
Methodology Code: Biospectroscopy
The practice of Raman spectrometry has been undergoing a continuing resurgence since 1986 when Hirschfeld and Chase first published their work on FT-Raman spectroscopic measurements. This work was followed by developments in the area of holographic filters for Raman, diode lasers, charge-coupled device (CCD) detectors, compact Raman microprobes, benchtop Raman instruments, and hand-held Raman instruments.

Recently, much research effort has been focused on surface-enhanced and tip-enhanced Raman spectroscopy. These approaches have produced exciting advances, in terms of the sample interface and applications, and have lead to Raman being exposed to historically unreceptive disciplines.

A perhaps un-sung area that is benefiting from Raman analysis is in-situ process analysis. There advances in lasers, filters, and spectrometers have promulgated research but have lead to minimal process acceptance. A major hurdle toward the acceptance of Raman in the process arena has been sampling. Textbooks imply Raman can be easily interfaced to a variety of chemical states however until recently only minimal work has been conducted on the development of process-robust interfaces. In this presentation developments in the area of solid’s sampling, gas-phase, and interfacing to green-based flow-chemistries will be given and their impact on quantitative process analysis will be highlighted.
Poly-3-alkylthiophene (P3AT) is a polymer consisting of a series of thiophene rings, each of which hangs an alkyl chain as a side chain. When the series of thiophene rings are ordered in plane, a long conjugated double bond is generated along the main chain, which works as a good absorber of visible light to make the material a p-type organic semiconductor, and it is a representative compound used for a solar cell. Since an organic semiconductor has a very small exciton radius, the compound should be laid in a thin film to effectively pass the excited electron to an electrode. Thus far, the analysis of the thin film has long been performed by using XRD, and molecular conformation and orientation in an amorphous part has been unclear. In the present study, p-MAIRS spectrometry has been employed to reveal the details of a thin film of P3AT after a careful choice of an appropriate infrared band for the analysis. As a result, the molecular orientation was readily controlled by changing the solvent used for dissolving the compound for the spin-coating process, which has clearly been revealed by infrared p-MAIRS spectrometry.

Keywords: Infrared and Raman, Membrane, Surface Analysis, Vibrational Spectroscopy
Application Code: Materials Science
Methodology Code: Vibrational Spectroscopy
The ocean, covering nearly three-quarters of the Earth’s surface, plays a large but highly uncertain role in affecting clouds and climate. Sea spray aerosols directly impact climate by scattering solar radiation and indirectly through nucleating clouds, offsetting a highly uncertain amount of the warming induced by greenhouse gases. Waves and winds produce bubbles which break at the surface of the ocean, enriching sea spray particles in a myriad of chemical species ranging from inorganic salts to proteins to lipopolysaccharides. In addition to being chemically complex, the resulting sea spray aerosols are comprised of multiple phases and morphologies. In an effort to advance our understanding of this important class of atmospheric particles, scientists in the Center for Aerosol Impacts on Climate and the Environment have developed new approaches for accurately generating chemical complexity in the laboratory which includes a new ocean-atmosphere facility equipped with breaking waves. This presentation will describe the challenges associated with replicating realistic sea spray in laboratory. The critical importance of establishing low background concentrations and using bubble size distributions representative of real breaking waves to generate the proper composition of sea spray aerosol (SSA) will be discussed. Novel single particle measurements and remaining challenges in determining how seawater composition impacts the composition of individual sea spray particles and the associated climate properties will be discussed.
Mineral dust and sea spray aerosols represent two important classes of particulate matter present in the Earth’s atmosphere. Although very different in chemical makeup, both of these classes of particles can transform in the environment through a variety of chemical processes as they undergo atmospheric transport including heterogeneous reactivity with trace atmospheric gases. In this talk, micro-Raman spectroscopy and other single particle methods are shown to be useful tools to study changes in the chemical and physical properties of individual mineral dust and sea spray aerosol particles. Several different examples will be given including cation redistribution within individual sea spray aerosol particles upon reaction with nitric acid, a comparison of reactivities of different sea spray aerosol particles with nitric acid and a comparison of reactivities for different components of mineral dust aerosol with organic acids.

Keywords: Environmental/Air, Raman, Surface Analysis, Vibrational Spectroscopy
Application Code: Environmental
Methodology Code: Molecular Spectroscopy
Particles containing mixtures of organic material and inorganic salts represent a major class of particles in the atmosphere. As the relative humidity cycles in the atmosphere from high to low values, these particles can go through a range of phase transitions including crystallization (i.e., efflorescence), dissolution (i.e., deliquescence), and liquid-liquid phase separation. An accurate understanding of these phase transitions is required when predicting climate and air quality. In the following we investigate liquid-liquid phase transitions in individual particles containing mixtures of organics and inorganic salts using optical and fluorescence microscopy. We show that for certain oxygen-to-carbon ratios these phase transitions occur at relative humidities between 95 and 40%.

**Keywords:** Aerosols/Particulates, Air, Environmental, Environmental/Air

**Application Code:** Environmental

**Methodology Code:** Microscopy
Atmospheric aerosols are a complex micro-structured mixture of inorganic and organic components, where organics can represent more than 50% of the aerosol mass depending on location. Understanding and predicting the climate effects due to atmospheric aerosols requires quantitative knowledge of their hygroscopic and chemical properties. The ability of aerosols to absorb water influences their optical and cloud forming properties, ice nuclei formation and transformation, atmospheric lifetime, and chemical reactivity. The presence of organic carbon in aerosols has a complex effect on their physical-chemical and reactive properties that is poorly understood. In particular, there is a lack of robust quantitative measurements on how properties of organic components (such as particle size, morphology, chemical composition, concentration and type of mixing) influence the rate and amount of water uptake on aerosols. Here an experimental approach based on a unique combination of Scanning Transmission X-ray Microscopy (STXM) and Atomic Force Microscopy (AFM) is presented for quantitative analysis of density, composition and hygroscopic properties of individual submicron particles. The approach utilizes AFM for direct determination of three-dimensional shape of substrate-deposited individual particles followed by STXM spectro-microscopy analysis at different relative humidity. Several field and laboratory generated atmospheric relevant inorganic and organic samples will be presented.
The rates of heterogeneous reactions of trace gases with aerosol particles are a complex function of particle chemical composition, morphology, and phase state. Currently, the majority of model parameterizations of heterogeneous reaction kinetics focus on the population average of aerosol particle mass, assuming individual particles have the same chemical composition as the average state. Here, we assess the impact of particle mixing state on modeled heterogeneous reaction kinetics using the dependence of the N2O5 reactive uptake coefficient $g(N2O5)$ on the particulate chloride-to-nitrate ratio as a model system. We describe the first simultaneous ambient observations of single particle chemical composition and in situ determinations of $g(N2O5)$. When accounting for particulate mixing state, model parameterizations of $g(N2O5)$ continue to over predict $g(N2O5)$ by more than a factor of two in polluted coastal regions, indicating that the chemical composition and physical phase state of particulate organics likely control $g(N2O5)$ in these air masses. In contrast, direct measurement of $g(N2O5)$ in air masses of marine origin are well captured by model parameterizations and reveal limited suppression of $g(N2O5)$ relative to its known value on NaCl particles, indicating that the organic mass fraction of fresh sea spray aerosol at this location does not suppress $g(N2O5)$. This study provides an observation-based framework for assessing the impact of particle mixing state on gas-particle interactions.

Keywords: Aerosols/Particulates, Environmental, Environmental Analysis, Environmental/Air
Application Code: Environmental
Methodology Code: Mass Spectrometry
X-ray photoelectron spectroscopy (XPS) is one of many tools available for surface characterization of a new generation of materials. The nearly 50 years of research and development has made XPS a powerful tool and the most widely applied capability for surface chemical analysis. XPS is most commonly used to detect the presence of a specific chemical species or the chemical state of an element. We use XPS to address a wide variety of scientific and technology challenges at our Environmental Molecular Sciences Laboratory (EMSL), a U.S. Department of Energy (DOE) scientific user facility. At EMSL, we collaborate with researchers from academic institutions, national laboratories and industry to provide solutions to energy, environmental challenges. EMSL's vast suite of analytical capabilities allows a wide variety of different experimental and computational methods to be combined. In particular, we have used advanced characterization techniques such as XPS in combination with various in-situ processing capabilities to gain deeper insights into mechanisms associated with chemical and material transformations in oxides, catalysts, nanostructured materials, and battery electrode surface films. Characterization of these air and moisture sensitive materials presents a variety of analysis challenges and some of these are associated with understanding how the materials respond in simulated environments.

We have established various in-situ processing capabilities and sample handling methods in combination with XPS so that the samples can be analyzed after processing under controlled environments. An overview of the principals of the technique, technological advances, capabilities, and methods will be presented along with the scientific highlights. Examples of routine and unusual sample handling, mounting, vacuum transfer methods for unique specimens will also be presented.

Keywords: Characterization, Electron Spectroscopy, Energy, Materials Science
Application Code: Materials Science
Methodology Code: Surface Analysis/Imaging
The understanding of the structural and chemical properties of oxides has become increasingly important due to their applications in several areas. In particular, there is a high demand for oxide thin films with good crystalline quality due to their applications in heterogeneous catalysis, magnetic thin films, high and low-dielectric materials, corrosion and integrated devices. High-energy ion scattering techniques have been extensively used to characterize these films. Although several high energy ion beam based techniques are available for material characterization, Rutherford backscattering spectrometry (RBS), nuclear reaction analysis (NRA), proton induced x-ray emission (PIXE), and elastic recoil detection analysis (ERDA) are commonly used. In general, the ion scattering spectroscopies are mass sensitive and mass selected information can be obtained from the data. As a result, backscattering and forward scattering along with nuclear reaction analysis capabilities are isotopic specific. In addition, since, ion beam can penetrate deeper into materials, RBS and other ion beam analysis methods can also be used to study the buried interfaces and diffusion profiles. Only drawback of the high-energy ion beam techniques is the lateral resolution is relatively poor. However, newly developed Helium ion microscopy (HIM) which utilizes low energy helium ions provides very high lateral resolution (resolution ~ 0.35 nm). HIM is very similar to scanning electron microscopy (SEM) but instead of using electrons as a probe beam, HIM uses helium ions. Since HIM uses an ion beam, it has several advantages over SEM. In this talk, we report on how effectively these ion beam techniques can be utilized to understand the mechanism of atomic disordering and growth of amorphous silica at the SrTiO3 single crystal film/Si interface due to oxygen diffusion from the film to the interface at elevated temperatures in vacuum, hydrogen and oxygen environments.

Keywords: Materials Characterization, Materials Science, Microscopy, Surface Analysis
Application Code: Materials Science
Methodology Code: Surface Analysis/Imaging
Information on material characteristics such as chemical composition, order (crystal phase, grain size, disorder), and sample structure (thin film thickness, interface formation, spatial non-uniformity, surface morphology) are extracted from spectroscopic ellipsometry measurements. All of these properties influence the behavior of thin films in devices (such as sensors and solar cells), as well as the device’s ultimate performance. Spectroscopic ellipsometry requires no additional sample preparation and can be used to measure layers in the device configuration or during fabrication. This data can be acquired over the near infrared to ultraviolet in less than one second per ellipsometric spectra, allowing for monitoring of changes occurring during processing or quickly scanning samples for quality control and assessing uniformity. Extension of the spectral range by Fourier transform infrared (FTIR) spectroscopic ellipsometry measurements provides additional sensitivity to the vibrational modes and conduction by free carriers, which can be connected to bonding configurations and electronic transport properties, respectively. In situ real time spectroscopic ellipsometry applied during thin film growth to track structural and compositional changes, ex situ mapping assessing spatial variations over large areas, and ex situ infrared extended measurements probing hydrogen bonding in amorphous silicon and carrier concentration of transparent conducting oxide layers within complete photovoltaic devices will be highlighted.

Keywords: Materials Characterization, Materials Science, Process Monitoring, Spectroscopy
Application Code: Materials Science
Methodology Code: Other (Specify)
Secondary ion mass spectrometry (SIMS) is a unique surface analysis technique because of its super-high sensitivity (down to ppb level), decent lateral resolution (down to 50 nm), as well as elemental/isotopic/molecular recognition capabilities. It plays a very important role in both semiconductor industry and scientific research. Traditionally, SIMS is a depth profiling tool with sub-ppm sensitivity and sub-nanometer depth resolution, making it the best tool to track trace doped elements in semiconductors. Recent years, applications of SIMS have been greatly expanded in two directions: 3-dimensional molecular imaging and 2-dimensional chemical imaging with nano-scale lateral resolution. These developments are majorly attributed to invention and improvement of two sophisticated SIMS instrumentations: ToF-SIMS and NanoSIMS. These two state-of-the-art instrumentations are available at Environmental Molecular Sciences Laboratory (EMSL), a national scientific user facility sponsored by the Department of Energy's Office of Biological and Environmental Research and located at Pacific Northwest National Laboratory. Collaborations are very welcome. In this presentation, history, principle, and major applications of SIMS will be discussed.

Keywords: Imaging, Mass Spectrometry, Surface Analysis
Application Code: Materials Science
Methodology Code: Surface Analysis/Imaging
The Blind Men and the Elephant as Metaphor for the Multi-Technique Analysis of Surfaces and Materials

In the fable of 'The Blind Men and the Elephant' each blind man touches one, but only one, part of an elephant -- one man touches its trunk, another its tusk, another its leg, etc., and each man obtains different information about the animal. Of course the whole picture is the sum of the stories of the individual men.

Unfortunately, there is no single analytical tool that can thoroughly characterize a surface or a material. Rather, like the blind men and the elephant, one often must rely on a suite of techniques to gain a deeper understanding of it.

For example, we have recently designed carbon nanotube (CNT)-templated thin layer chromatography plates that give extremely high plate counts and fast separations. These devices are prepared on silicon substrates that are then modified with ca. 35 nm alumina barrier layers, and ca. 6 nm iron layers, which are annealed to form Fe nanoparticles. CNTs are then grown on these surfaces, which in turn are conformally primed and coated with silica.

An appropriate understanding of these materials has come through a suite of characterization techniques -- X-ray photoelectron spectroscopy (XPS) for elemental analysis and oxidation state information, time-of-flight secondary ion mass spectrometry to complement the XPS analyses and provide molecular information, Rutherford backscattering for quantitative elemental analysis of the entire stack, spectroscopic ellipsometry to obtain the optical constants of the materials (useful for process development), atomic force microscopy and scanning electron microscopy to understand the nanoparticle formation, Helium ion microscopy to give very high resolution images of the materials, etc. Often, in addition to providing new information, the results from one analytical technique support those from another. Artifacts from a given technique can often be identified by using multiple methods.

A much clearer picture of each film and material in our new devices is obtained through the combination of the above mentioned surface analytical techniques.

Keywords: Characterization, Materials Science, Surface Analysis
Application Code: Materials Science
Methodology Code: Surface Analysis/Imaging
The airways of the healthy human respiratory have traditionally been considered to be “sterile”. However, the application of sensitive molecular detection methods combined with high throughput sequencing of bacterial 16S ribosomal RNA (16S rRNA) genes present in respiratory specimens has revealed a diversity of resident microbial species in the human lung. Disease and environmental exposure can alter innate airway defense mechanisms, resulting in intermittent and/or chronic bacterial infection. Understanding how alteration of innate immune mechanisms impacts the airway microbiome and ultimately human health is of substantial medical importance. We have previously shown that the airway microbiome of individuals with the genetic disease cystic fibrosis (CF) shows decreased diversity and increased bacterial abundance relative to normal individuals. Further, the composition of CF airway microbial communities appears to change with age and disease severity. To determine the impact of cigarette smoke and smoking induced chronic obstructive pulmonary disease (COPD) on the airway microbiome, we investigated three subject populations: 1) normal healthy subjects; 2) chronic cigarette smokers with normal lung function; and 3) subjects with varying severities of smoking induced COPD. Using a variety of invasive and non-invasive sampling techniques, we found that microbial diversity and composition are indicators of disease progression. Changes in the relative abundance of specific bacterial phylogenetic groups are associated with disease status. The lower airway microbiome appears to be largely overlapping with the oral microbiome; however, careful sampling methods indicate unique differences between these distinct but contiguous anatomical sites. In summary our findings indicate that the composition of airway microbial communities may serve as a surrogate measure of patient health.
Low molecular weight metabolites exist in many metabolic pathways, thus a single measurement of their concentration rarely yields specific information. Moreover, compared to large, non-volatile metabolites in e.g. blood analysis, the analysis of their volatile counterparts is more challenging due to potential losses during sampling and analysis.

We present two examples, where the exchange of volatiles from the liquid- into the gas-phase can elegantly be exploited to monitor volatile metabolites non-invasively and in real-time. Proton-Transfer-Reaction Mass-Spectrometers (PTR-MS) are to date the most sensitive instruments for real-time gas analysis of volatile organic compounds (VOCs). Their fast response time, soft ionization, and high sensitivity make them ideal tools to monitor the volatile metabolome.

In the first example, we study a simple metabolic system. In an industrial bioreactor genetically modified microorganisms produce modern drugs. The air-flow through the bioreactor provides oxygen to the cells and also takes up volatile metabolic byproducts. Using a PTR-MS, the volatile metabolome can be analyzed in the off-gas. In such a system all cells perform the same metabolic function and some emitted volatiles can be directly correlated to specific metabolic processes, yielding highly relevant information for process monitoring and control. The human volatile metabolome can be monitored similarly. The alveolar blood-gas exchange allows to analyze volatile metabolites in exhaled breath. The human body is a much more complex system and a direct correlation of a VOC to metabolism is mostly not possible. By administering isotopically labelled educts, the metabolic products will also be labelled and can clearly be distinguished from the metabolic background. This allows to probe and study specific metabolic processes. For both applications, monitoring the variation in time yields specific information from unspecific metabolites.

Keywords: Biopharmaceutical, Mass Spectrometry, Metabolomics, Metabonomics

Application Code: Biomedical
Methodology Code: Mass Spectrometry
Analysis of Microbiome Contributions to the Human Biomarker Metabolome

**Abstract Title**

A Critical Review on the Comparison of Volatiles in Breath, Urine, Blood, Milk, Saliva, Skin and a Comparison of Volatiles in Stool from Healthy and Diseased Human Volunteers

**Primary Author**

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**Abstract Text**

For the first time a data base of all the volatiles ever to be reported to be evolved from the healthy human body has been compiled by the author, with co-authors. This will benefit disease diagnoses. When we study volatile organic compounds (VOCs) from the human body eg breath or skin it is interesting to consider where the VOCs arise from. What are the similarities in VOCs from different parts of the body and what differences are there? This review will attempt to explain the sources of VOCs and the inter-relationship between them. The gastro intestinal tract is like a large organ, acting as a chemical factory producing a very wide range of volatile compounds covering numerous chemical classes of compounds, alcohols, ketones, esters, aromatic compounds eg furans, pyrroles etc, which to varying degrees can enter the blood stream where they can be chemically altered by the liver/other organs and then excreted by the lungs and into the urine via filtering through the kidneys. Volatiles can also be biosynthesised within the body. The lecture will cover changes in VOCs in the gut with gastro intestinal disease and whether they can be detected in stool urine or breath.

**Keywords:** Gas Chromatography/Mass Spectrometry, Specialty Gas Analysis, Metabolomics, Metabonomics

**Application Code:** Bioanalytical

**Methodology Code:** Gas Chromatography/Mass Spectrometry
We are developing an approach to rapidly (<30 seconds) profile bacterial infection as well as exposure to bacterial antigens. We use advanced mass spectrometry, particularly, secondary electrospray ionization-mass spectrometry, to profile the host-pathogen volatile metabolome (typically < 300 a.m.u.).

For this presentation, we demonstrate that breath from infected mice can be used to diagnose bacterial infection for seven major lung pathogens (Pseudomonas aeruginosa, Legionella pneumophila, Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae, Moraxella catarrhalis, and Klebsiella pneumoniae; P<0.001). In addition, SESI-MS was used to differentiate between chronic versus an acute infection strain (P<0.001) in the mouse. We also show that the infection breathprint can persist even after the infection has cleared. And, that this infection breathprint is different that elicited from antigen exposure. Taken together, this data suggests that it may be possible to rapidly identify the causative agent of bacterial infection as well as simply exposure to the pathogen, this improving diagnostics as well as providing a method for screening a population more effectively for infection during outbreaks.
The human exposome represents combined contributions from environmental exposure of exogenous compounds and microbiome-generated endogenous substances. Each individual will have a unique exposome – diverging to varying extents – due to past and immediate exposures, dietary intake, and the degree and diversity of microbial activity in the body. Taken as a whole, the exposome presents a challenge for medical diagnosis using biomarkers by introducing a range of possible confounding variables with the potential to veil true endogenous perturbations in the volatolome from disease. Focussing on diet, what we eat and drink has a notable impact on the volatile constituents of bodily media, initially in the blood and subsequently in excretion fluids such as urine, sweat and breath – amongst others – once the blood is filtered through the kidneys or interacts with inhaled gases in the lungs. Considering the latter, inhaled air will equally contain volatiles from the environment that can be taken up by the blood, distributed through the body, and similarly be excreted via the aforementioned routes. Metabolism also makes an important contribution to the exposome, since new compounds are potentially generated from exogenous precursors and might be erroneously classified as biomarkers due to their absence in the environment or diet. It is therefore imperative to investigate and thereby identify and collate compounds of exogenous origin before unique biomarkers can be used for diagnostic purposes with the necessary degree of reliability. This talk will report on exogenous volatiles from dietary intake, pharmaceutical administration and inhalation exposure detected in breath and urine. Investigations on bodily media were carried out using proton-transfer-reaction mass spectrometry (PTR-MS) and gas chromatography-mass spectrometry (GC-MS). The issue of pharmacokinetics of these substances, including degradation and metabolism into new products will also be addressed.

Keywords: Analysis, Gas Chromatography, Mass Spectrometry, Medical
Application Code: Environmental
Methodology Code: Mass Spectrometry
Live cell detection of RNA levels provides the ability to observe RNA expression within the active environment of a single cell. Furthermore, single cell resolution of RNA levels can highlight discrete events which can be masked within mixed populations when only analyzed at the population level. Understanding the individual responses to a given stimuli provides more biologically relevant information which can lead to a better understanding of population dynamics.

The ability to detect mRNA and miRNA levels within live cells without altering gene expression or affecting cell health allows for a better method of performing cellular screening assays. However, monitoring RNA within intact cells can prove to be challenging with current techniques due to complex or harmful sample preparation techniques or transfection reagents. Further, amplification methods can create false positives or erroneously inflate differences.

Here we present data showing gold nanoparticles conjugated with sequence specific oligonucleotides detect cytoplasmic RNA within live cells. The RNA detection was then paired with antibody staining resulting in simultaneous monitoring of mRNA and Protein within the same cell. Studying both protein and message at the single cell level in live cells is more precise than current techniques which rely on duplicate wells one for the RNA preparation of the population and the other for the protein detection.

Keywords: Biosensors, Genomics, Nanotechnology, Nucleic Acids
Application Code: Nanotechnology
Methodology Code: Fluorescence/Luminescence
### Applications of Live Cell RNA Detection

#### Detection of Circulating Tumor Cells Using NanoFlare Sensors

The NanoFlare is an oligonucleotide modified gold nanoparticle that enters cells and emits a fluorescent molecule ("the flare") upon binding to its target. We are developing NanoFlares as research tools for live cell circulating tumor cell (CTC) detection. A technician simply adds NanoFlares directly to a fractionated blood sample, and then analyzes RNA in individual cells via flow cytometry. The ability to collect and analyze cells in a straightforward manner and the ability to identify CTCs based on RNA expression will lead to a fundamentally new way of evaluating treatments of cancer.

**Keywords:** Biological Samples, Biomedical

**Application Code:** Biomedical

**Methodology Code:** Microscopy
Applications of Live Cell RNA Detection

Studying Tumor Cell Heterogeneity and Cancer Stem Cell Subpopulations

One of the critical issues in designing efficient cancer therapies is understanding the composition of heterogeneous tumors in order to target cancer stem cells and drug resistant subpopulations. Particularly challenging is metastatic melanoma, a disease whose incidence and mortality rates have been increasing over the last few decades, and is highly resistant to conventional chemotherapies. Our findings have revealed the re-emergence of a normally dormant Nodal embryonic pathway underlying melanoma stem cell plasticity, drug resistance, tumorigenicity and metastasis. Understanding the impact of this embryonic signal on tumor cell heterogeneity holds significant promise for new cancer therapies. However, until the recent development of SmartFlare™ Probes, we have been unable to study in real time the specific contributions of heterogeneous melanoma cells (expressing Nodal) with respect to stem cell plasticity and drug resistance -- with the overall goal of targeting these critical pathways in continuous experiments. Using SmartFlare™ Probes designed to detect Nodal, we performed live cell sorting of metastatic melanoma cells and grouped them into two experimental categories: Nodal-high expressors vs. Nodal-low expressors. These two subpopulations were then seeded into soft agar to assess clonogenic/tumorigenic potential. After three weeks in the soft agar assay, the Nodal-high expressors formed significantly more tumor colonies compared with the Nodal-low expressors. FAC analysis and confocal microscopy of these melanoma cells further revealed subpopulations of CD133 (associated with drug resistance)-expressing tumor cells within the broader subpopulation of Nodal-high expressing melanoma cells. Collectively, these data suggest a novel combinatorial approach should be considered in targeting heterogeneous metastatic melanoma cells expressing markers associated with cancer stem cells and drug resistance.

Keywords: Biological Samples, Biomedical, Detection

Application Code: Biomedical

Methodology Code: Other (Specify)
Evidence has grown in the last ten years in support of the cancer stem cell hypothesis. When an adult stem cell mutates to start a malignant growth, the resulting tumor presents as a heterogeneous population of cells and in most cases is refractory to current treatment methods. Within this mixed population of cells, there exists a small fraction of tumor cells that retain stem cell characteristics, such as the ability to self renew. These cells studied in vitro have shown extreme levels of resistance to currently used chemotherapy drugs and radiation. Current methods for isolating these cells have been less than robust. The literature shows a serious lack of consensus among scientists, using a wide variety of surface markers and functional assays to identify cancer stem cells. Commonly used markers include CD133, CD44hi/CD24lo, CD105, aldehyde dehydrogenase enzyme activity and side population sorting (MDR1 functional assay). Isolated cells must be further confirmed for the expression of universal stem cell transcription factors such as Nanog, Sox2, and KLF4. Present methods to detect these gold standard stem cell markers are destructive, analyzing either by PCR or intracellular Immunostaining.

Here we present data showing the use of a nanoparticle based live cell mRNA detection method and flow cytometry, to label and sort cancer stem cells based on the expression of Nanog, Sox2, and KLF4 mRNA transcripts from freshly digested human tumor samples. The technology works across a wide variety of solid tumor types and is compatible with surface Immunostaining. This method greatly simplifies the process of identifying and isolating cancer stem cells, providing a level of accuracy not previously available.
Messenger RNA (mRNA) is, by its nature, transient, beginning with transcription and ending with degradation, but with a period of processing and transport in between. As such, the spatiotemporal dynamics of specific mRNA molecules are difficult to image and detect inside living cells, and this has been a significant challenge for the chemical and biomedical communities. To solve this problem, we have developed a variety of molecular probes for intracellular mRNA monitoring. The first one is a targeted, self-delivered, and photocontrolled aptamer-based molecular beacon (MB) for intracellular mRNA analysis. The second one is DNA micelleflares for effective delivery of nucleic acid probes into intracellular environments. We will discuss these molecular probes and their applications for intracellular measurements.

Keywords: Biosensors, Clinical Chemistry
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Sensors
Abstract Text
Sol-gel processing is widely used to create low-k materials, thermal insulations, and stationary phases in the separation sciences. Xerogels, porous sol-gel derived materials formed by solvent evaporation at or near ambient conditions, are also attractive platforms for chemical sensor development. Over the past 20 years our research group has devoted significant time and effort to elucidate the chemistry within amorphous silica-based xerogels as a means to intelligently guide the development of useful xerogel-based materials for use in areas ranging from chemical sensors to anti-fouling coatings. More recently we have been coupling thin films of amorphous silica-based xerogels to photoluminescent nanocrystalline materials (e.g., silicon quantum dots and porous silicon) as a way to create nanoscale analyte-responsive sensors. The speaker will summarize his research team’s pathway to developing hybrid silica-silicon nanosensors.
In the work here, we describe sensing motifs that utilize 2-D and 3-D arrays and monolayers of particles embedded onto a molecular recognition polymer hydrogel network. The 2-D arrays alter their visually evident diffraction color because the hydrogel network swells or shrinks in response to analyte concentration changes. We developed two-dimensional (2-D) photonic crystals for molecular recognition and chemical sensing applications. We prepared close packed 2-D polystyrene particle arrays by solvent evaporation of an assembling monolayer on a mercury surface. We then transferred the 2-D arrays onto a hydrogel thin film that showed a hydrogel volume phase transition in response to a specific analyte. This altered the array spacing, changing the array diffraction wavelength. These 2-D array photonic crystals exhibit ultrahigh diffraction efficiencies that enable them to be used for visual detection of changes in the analyte concentration. The 3-D arrays undergo 3-D volume changes to shift the diffraction wavelength.
Design and Application of Smart Materials for Chemical Sensing and Analysis

Fluorescent and Photoacoustic Based Nanosensors for In Vitro and In Vivo Chemical Analysis

Biomedical nanosensors have been developed and extensively utilized for real-time imaging and dynamic monitoring of chemical properties, response to external stimulants and metabolism of cells and tissues, in real time, using optical imaging techniques. Such an indicator dye based optical sensor nano-PEBBLE (Photonic Explorer for Bioanalysis with Biologically Localized Embedding) usually employs polymeric, silica and ormosil matrix. The advantages of having a matrix include: (1) minimal perturbation of cell components on the indicator dye, (2) minimal chemical perturbation of the dye on the cell, (3) targeting to specific cell locations and (4) ratiometric and synergistic operation principles. A large fraction of the polymeric nanoparticles are based on polyacrylamide (PAA) due to its excellent properties such as non-toxicity, biocompatibility, biodegradation, bioelimination and flexibility of engineering. The properties of the PAA matrix can be specifically tailored, depending on the application, and the molecules can be loaded into the matrix. Various surface modifications enable one to control its behavior in cells and in vivo, and can be utilized for specific targeting to cells as well as to subcellular organelles. Recent advances in the design and application of these nanosensors have concentrated on physiologically important ions, radicals and small molecules. In vivo applications, in mice and rats, have mainly been carried out photoacoustically. These involve tissue penetrating, time-resolved, infrared laser excitation, followed by acoustic detection. In this case we use the absorbance property of the dye, rather than its luminescence, i.e. indicator dyes with the lowest quantum efficiencies are optimal. Thus photoacoustic nano-PEBBLE sensors appear to be ideal for in vivo chemical analysis.

Keywords: Fluorescence, Nanotechnology, Photoacoustic
Application Code: Other (Specify)
Methodology Code: Other (Specify)
Analytical methods that would enable accurate and continuous monitoring of blood glucose levels would dramatically alter the clinical management of diabetes. Unfortunately, the foreign body response (FBR) to sensor implantation has limited the utility of electrochemical biosensors for continuous glucose monitoring. The polymer composition of the biosensor that contacts interstitial fluid and tissue has been shown to directly impact the severity of the FBR and concomitant reduced sensor performance. Herein, the fabrication of electrospun fiber-coated nitric oxide-releasing glucose sensors will be introduced as a strategy for improving tissue integration and sensor performance based on passive and active release strategies.

**Keywords:** Biomedical, Biosensors, Electrochemistry, Polymers & Plastics

**Application Code:** Biomedical

**Methodology Code:** Sensors
Solid-phase extraction (SPE) is a well-known method for separating and preconcentrating analytes prior to ex-situ quantification. Despite advances in miniaturization of SPE, the technique has not kept up with a need for detection in small scale microfluidic and biological systems. One reason efforts have remained unsuccessful is that while micro- and smaller scale extractors allow for preconcentration from small volumes, detection in correspondingly small volumes has remained out of reach because of analyte dilution during post extraction transfer or “wash off” steps prior to detection. In this work, SPE into individual femtoliter-sized particle extractors is accomplished by using confocal Raman microscopy for in-situ detection within the collection phase, thus avoiding wash-off dilution. Specifically, a stable pH gradient established across the membrane of phospholipid vesicle can induce $10^5$-fold accumulation of ionizable compounds from bulk solution into the vesicle interior. This process is also exploited to preconcentrate dilute analytes from free solution into phospholipid vesicles, followed by detection using confocal Raman microscopy. In addition, nanomolar concentrations of polycyclic aromatic hydrocarbons in aqueous solution can be concentrated into single C$_{18}$-functionalized silica or vinyl-polymerized surfactant particles, and spectra in a small volume (~1fL) within the interior of the particle are acquired. Despite the in situ nature of these measurements, the results can be quantitative through the comparison of Raman scattering intensity of an analyte with that of an internal standard in vesicle, or in the latter cases, the n-alkane chains of the surface C$_{18}$ groups or surfactant tails. Thus, it is possible to determine the concentrations of analytes within a particle, both to characterize the equilibria on a single-particle basis and to infer the low concentrations in the source phase.

**Keywords:** Materials Science, Microspectroscopy, Raman, Solid Phase Extraction

**Application Code:** Nanotechnology

**Methodology Code:** Vibrational Spectroscopy
This presentation briefly reviews the capabilities of various MS imaging methods, particularly those of DESI. The body of the talk covers DESI imaging applications such as microorganism cultures, intact plant tissue and diseased animal (and human) tissue. The ability to identify metabolic products under a variety of stimulation conditions is shown using DESI imaging and a simple ion trap mass spectrometer capable of MS/MS. The use of DESI to follow the early stages of embryological development through spatially and temporally resolved lipid profiles is illustrated in some detail using recent examples emphasizing the impact of in vitro culture systems. DESI-MS has been shown to provide comprehensive analytical information on small molecules (especially lipids) from complex microscopic and valuable biological samples. Analysis of organisms/cells end-point metabolism by DESI-MS can be used for bottom-up (DESI-MS data used to guide proteomics, transcriptomics or genomics analysis) or top-down metabolomics (DESI-MS data is collected to confirm and detail pathways indicated by other omics methods). A third example seeks to distinguish various stages of cancer in dogs and humans. Data shows that powerful, fast and informative small molecule analysis by DESI is provided from complex biological models. This approach is especially interesting if limited amounts of sample are available and/or a large number of samples must be analyzed. DESI-MS represents end-point metabolic analysis and can be integrated into other omics approaches. These capabilities depend heavily on advanced bioinformatics methods which are also discussed.
Imaging mass spectrometry (IMS) provides an attractive opportunity to detect and probe the molecular content of tissues in an anatomical context. This technique creates distribution maps of select compounds without the need for priori knowledge of target analytes. This presentation will describe our efforts and recent progress in mapping and imaging of various signaling molecules in crustacean and mammalian nervous systems, highlighting the unique challenges and important roles of IMS in the areas of neuropeptidomics and metabolomics. Sample preparation is essential for obtaining sufficient analyte/matrix mixing while retaining the spatial localization of analytes of interest. Both 2D and 3D spatial imaging of neuropeptides will be highlighted. To address the unique challenge of small molecule imaging due to MALDI matrix interference, we employed a high-resolution and high-accuracy mass spectrometer, MALDI LTQ Orbitrap, to allow differentiation of endogenous analytes from matrix peaks. The high mass-accuracy and spectral resolution of MALDI Orbitrap enabled unambiguous, confident identification of numerous metabolites and neurotransmitters directly from tissue samples. Furthermore, the combination of orbitrap full scan and multiplex targeted tandem MS imaging of neuropeptides greatly enhanced chemical information one can extract from limited biological specimens. Lastly, although MALDI IMS is typically employed in tissue imaging, we explore its novel coupling with various micro-separation techniques, such as capillary electrophoresis (CE) or nanoflow HPLC. IMS enables “continuous” collection and detection of CE or LC flow which preserves electrophoretic or chromatographic resolution. Moreover, CE or LC provides separation and quantitation ability for IMS, offering a unique tool for enhanced peptidomics.

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

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
The plasma membranes of mammalian cells consist of hundreds of different lipid and protein species. However, cohesive interactions between just a few components, namely cholesterol and sphingolipids, are widely thought to be responsible for organizing the plasma membrane into compositionally and functionally distinct domains. Despite their potential importance to mammalian cell function, few methods exist for directly imaging the distributions of cholesterol, sphingolipids, and most other lipid species in cell membranes with high lateral resolution without the use of potentially perturbing labels. Therefore, we have developed a high-resolution secondary ion mass spectrometry (SIMS) approach for chemically imaging the distributions of isotope-labeled cholesterol and sphingolipids in the plasma membranes of intact cells with high (<100 nm) lateral resolution. In this approach, distinct stable isotopes are metabolically incorporated into lipid species of interest. Then the lipid-specific isotope enrichment is mapped with high-resolution SIMS. By using this approach to image the distributions of metabolically incorporated $^{15}$N-sphingolipids and $^{18}$O-cholesterol in the plasma membranes of fibroblast cells, we found that sphingolipids, but not cholesterol, is enriched within distinct domains in the plasma membrane. We have also used this approach to investigate the hypothetical roles of favorable cholesterol-sphingolipid interactions and the actin cytoskeleton in establishing nonrandom lipid distribution with the plasma membrane. We show that lipid organization in the plasma membrane is heavily dependent on the cytoskeleton, but only moderately influenced by cholesterol depletion.

Keywords: Bioanalytical, Imaging, Lipids, Surface Analysis
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Imaging Mass Spectrometry of Biological Tissues and Cell Cultures

Silver Assisted LDI for High Spatial Resolution Imaging MS of Olefins from Thin Tissue Sections: Application to Atherosclerosis

In the past decade, cholesterol has been targeted by many studies for its important role in diseases, including Alzheimer, cancer, and atherosclerosis. Based on previous findings involving silver cationization of cholesterol we are exploring the analysis of olefinic compounds directly from tissue sections through metal cationization after silver sputtering by high resolution LDI imaging MS. For this purpose, various coating thicknesses of metallic silver were investigated on tissue sections from different mouse organs including brain, kidney, liver, and testis. The high spatial resolution (~5 µm) potential of this approach is also being explored. The method provides a rich molecular pattern, in the fatty acid and sterol range of 330-500 Da and the phospholipid range of 600-1000 Da. Using this desorption/ionization approach, imaging MS was carried out on mouse brain and kidney sections at 100 and 150 µm lateral resolutions respectively. The resulting ion images for cholesterol in mouse brain sections showed a high correlation with white matter structures such as the corpus callosum which is in accordance with the literature. Arachidonic acid and docosahexaenoic acid resulting images both showed high signal intensities in the front lobe and the hippocampus region but signal depletion is observed in the corpus callosum. High resolution imaging MS was also performed on a mouse cerebellum section for cholesterol at a lateral resolution of 5 µm. The resulting ion image showed a well-defined cerebellar lobule with high signal intensity surrounded by a low intensity region corresponding to the granular region and the cerebellar cortex with medium signal intensity. Silver assisted LDI 3D imaging MS is also being developed to monitor the spatial organization of cholesterol, fatty acids, triglycerides and phospholipids in atherosclerosis plaques. Recent findings highlight region-specific cholesterol accumulation and spatial organization as a function of plaque development.

Abstract Text

In the past decade, cholesterol has been targeted by many studies for its important role in diseases, including Alzheimer, cancer, and atherosclerosis. Based on previous findings involving silver cationization of cholesterol we are exploring the analysis of olefinic compounds directly from tissue sections through metal cationization after silver sputtering by high resolution LDI imaging MS. For this purpose, various coating thicknesses of metallic silver were investigated on tissue sections from different mouse organs including brain, kidney, liver, and testis. The high spatial resolution (~5 µm) potential of this approach is also being explored. The method provides a rich molecular pattern, in the fatty acid and sterol range of 330-500 Da and the phospholipid range of 600-1000 Da. Using this desorption/ionization approach, imaging MS was carried out on mouse brain and kidney sections at 100 and 150 µm lateral resolutions respectively. The resulting ion images for cholesterol in mouse brain sections showed a high correlation with white matter structures such as the corpus callosum which is in accordance with the literature. Arachidonic acid and docosahexaenoic acid resulting images both showed high signal intensities in the front lobe and the hippocampus region but signal depletion is observed in the corpus callosum. High resolution imaging MS was also performed on a mouse cerebellum section for cholesterol at a lateral resolution of 5 µm. The resulting ion image showed a well-defined cerebellar lobule with high signal intensity surrounded by a low intensity region corresponding to the granular region and the cerebellar cortex with medium signal intensity. Silver assisted LDI 3D imaging MS is also being developed to monitor the spatial organization of cholesterol, fatty acids, triglycerides and phospholipids in atherosclerosis plaques. Recent findings highlight region-specific cholesterol accumulation and spatial organization as a function of plaque development.

Keywords: Imaging, Laser Desorption, Mass Spectrometry, Time of Flight MS
Application Code: Biomedical
Methodology Code: 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. Currently, we are interrogating the spatial distribution of proteins following the loss of function of the protein E-cadherin, a critical regulator of the metastatic process. Given the flexibility of cell culture, we can manipulate E-Cadherin expression and monitor the spatial changes in protein expression and phenotypic alterations that accompany E-Cadherin knockdown. We have also developed an approach to employ 3D cell cultures to evaluate the penetration of compounds into cellular masses. Most novel drugs are initially evaluated with 2D cultures before moving directly to costly animal studies. 3D cultures 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. In collaboration with Paul Bohn’s laboratory, we are also examining the drug penetration patterns and endogenous molecular changes by both MALDI imaging mass spectrometry and confocal Raman microscopic imaging. Our future studies include evaluation of drug and imaging probe libraries to evaluate the functional moieties that contribute to penetration of compounds, including the development of novel statistical workflows to evaluate imaging data generated from 3D cell cultures.

Keywords: Biological Samples, Imaging, Mass Spectrometry, Proteomics
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Analysis of single cells provides powerful insight into biological processes that are often missed when a population of cells is studied as an ensemble. We are developing microfluidic-based approaches coupled with optical microscopy to track individual bacteria and to improve the temporal and spatial resolution of single-cell measurements. To streamline our experiments, we designed and tested a microfluidic “baby machine,” which automates the culture, synchronization, and analysis steps. This microfluidic device is used to produce synchronized populations of cells and monitor adhesion of individual bacteria to surfaces. To better understand how bacteria attach to surfaces, rates of attachment of mutant strains that lack pili and motility are compared to the rate of attachment of wild-type cells. On-chip synchronization requires that cells adhere to the microchannel surface. To synchronize non-adherent cells and study cellular growth, we integrated nanochannel arrays into the microfluidic devices that physically trap bacteria. With these nanochannel arrays, we are able to study a number of bacteria strains and observe their aging and growth over multiple generations. Consequently, we are able to determine rates of cell division and accumulation of cellular damage as the bacteria age.

Keywords: Bioanalytical, Biological Samples, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Insights into epigenetics and chromatin dynamics have profoundly affected our understanding of biological processes including development, aging, complex diseases and oncogenesis, providing a more comprehensive view than could be ascertained by considering information from genetic or gene expression studies alone. Detection of protein complexes and histone modifications implicated in chromatin remodeling is crucial for developing new diagnostic and therapeutic strategies. However, chromatin immunoprecipitation (ChIP), the method of choice for investigating interactions between histones, non-histone proteins, and DNA in this context, is relatively complicated compared to tools used to identify and analyze somatic mutations and has yet to be standardized for clinical use. Briefly, ChIP entails crosslinking interacting proteins and nucleic acids (mainly required for studying loosely associated proteins), chromatin isolation, fragmentation to achieve resolution, immunoprecipitation of target proteins, dissociation of precipitated complexes, and recovery and analysis (e.g., by PCR or sequencing) of nucleic acids. The main limitations of the technique are (i) the requirement of large cell populations which precludes the analysis of tissue biopsies or other sparse samples, (ii) time and labor intensive procedures, and (iii) very limited throughput. To circumvent these restrictions, we are engineering a microfluidic system to carry out quantitative chromatin analysis, beginning with chromatin fragmentation and proceeding to solid-state immunocapture. We refer to the platform as micro-chromatin immunocapture, or ChIC. Importantly, the implementation of microfluidic circuitry will allow us to manipulate small sample volumes with high precision, integrate and automate the sample preparation and immunocapture steps, and perform multiple immunocapture steps in parallel. The resulting platform is expected to have broad utility in advancing sample-limited epigenetic profiling.
Early microfluidic and lab-on-a-chip devices were primarily fabricated in glass and silicon materials involving multistep procedures for completion. In the late 90’s, reports describing soft lithographic methods initiated a new trend in polymeric-based devices that required less sophisticated equipment for fabrication; however, these devices were still fabricated using multistep procedures. Here, data will be presented that was obtained using microfluidic devices that were fabricated using 3D printing technologies. Our devices fabricated with the 3D printer enable design-to-print capabilities with no wet-lab steps during fabrication. The devices are rugged, contain multiple materials, can be placed directly into a plate reader, and enable simple connections to pumps. The devices can also be created to be transparent. To date, our group has used these devices to create in vitro pharmacokinetic profiles of antibiotics. Data will also be shown that was generated using microelectrodes that can be screwed into the device, taken out, cleaned/polished, and then placed back in for re-use. We have also pumped complex samples such as blood. Advantages and disadvantages of these devices will be discussed, as well as their potential for future applications.
Exposure to airborne particulate matter (PM) is one of the leading causes of morbidity and mortality worldwide. The World Health Organization ranks PM exposure as the 9th leading cause of death, ahead of all other forms of environmental exposure. PM exposure has wide ranging health effects, causing diseases ranging from cancer to asthma attacks. Presently exposure assessment relies on large-scale community level measurements using stationary samplers because it reduces the overall measurement burden. Measurements of chemical composition analysis are normally done using traditional laboratory instrumentation resulting in analyses that are slow and expensive. PM exposure is highly dependent on individual activities and locations meaning that stationary samplers capture only a small picture of the overall exposure scenario. As a result, new portable tools for sampling and analysis are needed that allow for personal exposure assessment. This talk will focus on recent work in our laboratory focused on developing microfluidic paper-based analytical devices for analysis of pollutants in aerosolized particulate matter at the personal level. Paper-based analytical devices provide a low cost and readily portable solution to making personalized exposure measurements. Results for exposure to aerosolized metals in a factory as well as oxidative stress induced by indoor and outdoor air will be discussed. Integration colorimetric and electrochemical detection in a single device will be shown as a way to increase the number of analytes detected as well as the lower limit of detection.

Keywords: Electrochemistry, Environmental Analysis, Lab-on-a-Chip/Microfluidics
Application Code: Environmental
Methodology Code: Microfluidics/Lab-on-a-Chip
Recent work from our lab has involved the use of epoxy and polystyrene-based encapsulation of materials to integrate fluidic interconnects and electrochemical detection with other processes such as cell culture and microchip electrophoresis. This general encapsulation approach allows the electrode material to be polished before each use and also enables the use of different electrode materials. This includes a palladium electrode for coupling microchip electrophoresis with electrochemical detection as well as any working electrode material of choice (such as carbon or platinum). This talk will show some of this recent work and demonstrate how the polystyrene devices are much more robust for both the immobilization of cells (both PC 12 and endothelial cells) and the analysis of molecules (such as catecholamines and nitric oxide) released from these cells upon stimulation. A unique feature of this approach is the ability to create planar membranes from 3-dimensional pillar electrodes. Work towards using this type of device to monitor cell-to-cell interactions will be discussed. In addition, the ability to make low dead volume connections with the encapsulation approach will be described.

Keywords: Bioanalytical, Capillary Electrophoresis, Electrochemistry, Lab-on-a-Chip/Microfluidics

Application Code: Bioanalytical

Methodology Code: Microfluidics/Lab-on-a-Chip
Terahertz Technology for Safety and Security in Daily Life

The technology of terahertz (THz) electromagnetic waves has been developed greatly in this century. The technology for generating wideband THz wave pulses from photoconductive antennas or nonlinear optical crystals excited by near-infrared femtosecond lasers has led the modern THz technology. In addition, compact monochromatic THz sources using semiconductor technology have also been developed. THz cameras are developed as imaging devices. Spectroscopic and imaging systems are commercially available now.

THz waves are transmitted through plastics, papers, and ceramics considerably. Many molecular crystals have fingerprint absorption in the THz region. Since the wavelengths of THz waves are sub-millimeters, the spatial resolution in imaging is less than one millimeter. By using these properties, it is possible to detect hazardous materials in mails such as forbidden drugs and plastic bombs without opening them. Since THz waves are transmitted through thin plastics, inflammable liquids in plastic bottles can be discriminated from water based on the difference of the transmittance. Efficiency of a medicine is determined not only by its molecular structure, but also by its configuration in the crystal (polymorph). The THz spectrum is very sensitive to the polymorphs. By using THz pulses, the inner structure of objects can be investigated similarly to ultrasonography. They can be used to detect defects of covering of high-voltage cables. The THz technology can be also applied to art conservation, criminal investigation, etc. The fields to which the THz technology can be applied are expanding.

Keywords: Biomedical, Pharmaceutical, Polymers & Plastics, Vibrational Spectroscopy

Application Code: Safety
Methodology Code: Other (Specify)
MeV Gamma Imaging by Fully Reconstructing Compton Scattering

For MeV gamma-ray astronomy, we have developed Electron Tracking Compton Camera (ETCC) consisting of a Gas counter for tracking a recoil electron and pixel scintillators for a scattered gamma ray since 2001. By measuring the direction of an electron, the direction of the gamma ray is uniquely determined, which gives a good background rejection and a clear image. Already we revealed the excellent background rejection and imaging in the balloon experiment and the medical imaging. Since ETCC provides images for wideband gamma rays with a FoV of 3str, it has also very large potential for environmental monitoring and homeland security. Although there looks no established imaging method for MeV gamma rays, ETCC can reconstruct fully the Compton process for gamma rays, and hence a wide band energy gamma rays can be imaged. In 2013, we have improved the tracking method in Gas tracker based Time projection chamber, and now all tracks of the recoil electron in Compton scattering can be reconstructed. This improvement enables to select Compton events contained in TPC using only the energy loss rate in the recoil electron with distinguishing it from cosmic rays, neutrons and high energy recoil electron escaping from TPC. This simple method to select clear Compton event extracts the maximum detection efficiency expected for gas in the TPC. 10x10x15cm ETCC with Xe 3tm is expected to give a detection efficiency of over 3x10-3 at 662keV, which give a clear gamma-ray intensity map under the weak radiation condition of 0.1microSv with ten minute observation. In addition, the direction of a recoil electron makes clear image dramatically, where its contrast is improved several times. Also ETCC has already been operated under the intense radiation condition of proton therapy imaging and nuclear medicine. In addition, the direction of the recoil electron improves unclearness of Compton images dramatically. Thus, ETCC overcomes the serious problems of conventional Compton Camera, and thus open the new applications to not only astronomy but also medical and environmental imaging.

Keywords: Detector, Medical, Nuclear Energy, Spectroscopy
Application Code: Homeland Security/Forensics
Methodology Code: High-Energy Spectroscopy
For decades, scintillation materials with fast response are required for applications in which excellent timing resolution or high counting rate measurements are necessary. Conventionally, novel scintillation materials have been developed by using rare-earth ions as luminescence centers. In this approach, however, there is a severe limitation that a fast response should be achieved by a limited number of the rare-earth elements. Thus, in order to achieve a faster response, a novel fast scintillation material should be developed that does not use the rare-earth ions. On the other hand, plastic scintillators have been used for such applications. However, the plastic scintillators have their limited applicability, because they have very low interaction probability with high-energy photons and neutrons, resulting in poor detection efficiency.

In this research, we have developed novel scintillation materials by using materials having nanometer-scale structure. The advantage in using the nanometer-scale structure lies in the following two points;
(a) Free exciton luminescence from the semiconductor nanostructure can be used. Free exciton luminescence lifetime in direct-gap semiconductors is in the subnanosecond to nanosecond range. Thus, fast scintillation materials can be made of direct-gap semiconductors. However, that has been revealed to be quite difficult, mainly due to instability of the free excitons at room temperature. In order to overcome this difficulty, we have used quantum confinement effects.
(b) The detection efficiency of the plastic scintillators can be enhanced by fabricating plastic-oxide nanocomposite materials. In this approach, the scintillation materials can be separated into two parts; one is the plastic scintillator domain, and the other is oxide domain with which high-energy photons or neutrons interact. Besides, transparency of the materials to the scintillation light can be achieved by fabricating the composites in nanometer scale.

Keywords: Luminescence, Materials Science, Nanotechnology, Sensors
Application Code: Nuclear
Methodology Code: Sensors
Automated Nuclear Emulsion Readout System and Its Applications

Nuclear Emulsion is a three-dimensional radiation detector, which can record high energy charged particles and ions at sub-micron resolutions. We have developed an ultra fast readout system to analyze large amount of nuclear emulsion plates in the field of particle physics. This allows ten thousand times faster analysis speed compare to a worker with a classical microscope. Besides particle physics, nuclear emulsion and our readout system should be applicable to various field, one of which is Muon radiography. It can see through very large objects such as volcano to investigate internal structure by using cosmic-ray muon instead of X-ray for roengenograph. Since cosmic-ray muon flux is limited, larger area muon detectors are required, of which area is typically square-meter. Nuclear emulsion plate provides a high precision and compact detector which needs no power supply at low cost.

Keywords: Imaging, Microscopy
Application Code: Other (Specify)
Methodology Code: Other (Specify)
In previous sessions in this symposium, we described the role of ambient ionization mass spectrometry (in particular using the DART ion source) for chemical analysis problems related to safety and security. DART has continued to play a growing role in dealing with emerging problems. We have also found new ways to use DART and to combine it with other rapid-analysis techniques to extend the range of analytes that can be measured.

In recent years, the designer drug problem has grown to monstrous levels. Cayman Chemical, a supplier of standards to the forensic community, lists 549 cannabinoid standards and 108 cathinones in its catalog. New threats are appearing every day, challenging the ability of analytical chemists to keep up. DART is effective for rapid screening of designer drugs in so-called “herbal incense” and “bath salts”. Many of the new drugs are made by varying substituents attached to a set of common core structures. Exact-mass data and fragment-ion information can therefore provide information about substructures present in new drugs that have previously been reported. Combined data from mass spectrometry and NMR can determine the structure of new compounds for which standards are not available.

Mass spectrometry also plays an important role in food safety and environmental analysis. DART data in combination with chemometric tools show promise for oil typing and identification of biodiesel feedstocks, and the same approach has been applied to the identification of products from endangered species that are banned for importation.

Recently, we have begun to explore the environmental applications of GC x GC interfaced to a high-resolution time-of-flight mass spectrometer. These data provide detailed information about peaks observed in DART mass spectra. The GC x GC/HRTOF system is also very powerful tool for the identification of trace contaminants, such as pesticides, in complex mixtures.

Keywords: Environmental Analysis, Forensics, GC-MS, Mass Spectrometry
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
Solid-phase extraction (SPE) is an important tool required for the enrichment and purification of targets from complex samples. Micro-total analysis systems (uTAS) based on the use of microfluidics are becoming attractive platforms for the analysis of molecular targets with SPE typically used on the frontend to enrich the molecular targets and remove potential interferences. Analytical modalities for SPE require either the use of the appropriately prepared beads, such as silica particles packed into microchannels or polymer monoliths photochemically assembled in microchannels. We will discuss a SPE microfluidic based on the use of thermoplastics that can be molded into a variety of microstructures to allow the high-scale production of devices at low cost, appropriate for in vitro diagnostics. The SPE device consists of a microchannel populated with high-aspect ratio microposts (10 µm diameter; 50 µm tall). The SPE devices could be easily fabricated using any polymer substrate (poly(methylmethacrylate), PMMA, or polycarbonate, PC) from mold masters using molding. For nucleic acids, PC was used as the substrate and following exposure of the posts to UV light (254 nm), genomic DNA could be extracted from cell lysates with a recovery of ~70% using an immobilization buffer consisting of polyethylene glycol and NaCl. The PC SPE bed could also be used for the purification of total RNA by simply changing the composition of the immobilization buffer. The micropost SPE bed could be configured to extract membrane proteins from cell lysates by decorating the posts with avidin. Intact cells could be biotinylated and following lysis, the biotinylated membrane proteins enriched from the lysate with minimal amounts of cytosolic protein infiltration. These SPE beds could also be integrated to uTAS for securing high quality molecular information from cell lysates. As an example, the analysis of sputum for determining the presence of mycobacterium tuberculosis will be discussed.

Keywords: Bioanalytical, Chromatography, Sample Preparation
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Microfluidic technology has made significant inroads in analytical chemistry, with systems for analyzing a wide variety of samples and compounds being reported. Sample concentration and other sample preparation steps, reaction monitoring, immunoassays, sensor technologies and separations methods have all been reduced to practice in microfluidic formats. With regards to separation technologies, however, the bulk of these have been based on capillary electrophoresis, with the more widely applicable HPLC systems comprising a greater challenge for designing high performing devices. Over the past several years we have been developing an ultra high pressure capable microfluidic HPLC system based on high temperature co-fired ceramics with performance equivalent to standard packed capillary columns, yet offering the advantages of a microfluidics format. The optimized system operates at pressures up to 10 kpsi, and can be packed with a variety of stationary phases. Sample loading can be via direct injection or with a custom designed trapping system. Device cartridges can be readily changed without using tools and have built-in electronics for temperature control and other functions.

Prototype devices have been particularly useful for high sensitivity peptide analysis including biomarker identification, analysis of bioactive peptides in biofluids and protein bioanalysis via signature peptide quantification. Lipidomics and other metabolomics analyses have also proven to be highly advantageous with the microfluidic system. These assays and other small and large molecule applications will be described to illustrate key system attributes.

Keywords: Bioanalytical, Lipids, Liquid Chromatography/Mass Spectroscopy, Peptides
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
We have developed microfluidic systems that combine multiple on-chip sample preparation processes with subsequent electrophoretic separation. These miniaturized systems are automated and use small volumes of samples and reagents, offering excellent potential for rapid biomarker analysis. We have utilized on-chip immunoaffinity extraction to selectively purify several cancer-linked protein biomarkers from blood serum samples; following this step, the biomarkers were eluted into a microchip injection cross on the same device and separated electrophoretically [1]. We have further implemented solid-phase extraction with reversed-phase columns in microfluidic devices to selectively enrich protein samples. We then labeled these analytes while they were still retained on the column and eluted the unreacted fluorescent tag in a separate fraction from the labeled protein [2]. We are presently working to combine the processes of immunoaffinity extraction, preconcentration through solid-phase extraction, on-chip fluorescence labeling, and electrophoretic separation of the products in an automated and miniaturized platform. Such integrated sample preparation systems should enhance rapid biomarker analysis capabilities.

References

Keywords: Capillary Electrophoresis, Chromatography, Lab-on-a-Chip/Microfluidics, Solid Phase Extraction
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
For many years, the development of electrochromatography on chip has been slowed down because the synthesis of the stationary phase inside a microfluidic device was a difficult, non reproductive task. Recently, we have demonstrated that it is possible to synthesize an acrylate monolith by photopolymerization in cyclic olefin copolymer chip, with high reproducibility and perfect control of both structure and anchoring. This presentation will highlight the simplicity of fabrication of such a separation device as well as its simplicity of use. Exciting reversed-phase chromatographic performances on chip are illustrated by high efficiencies and fast analysis. This monolith-based electrochromatography device exhibits many interesting features such as strong resistance to aggressive conditions (organic solvents, extreme pH), application to a wide range of analytes (non-polar or polar, neutral or charged) and ability for on-line preconcentration.

Keywords: Lab-on-a-Chip/Microfluidics, Liquid Chromatography, Separation Sciences
Application Code: Other (Specify)
Methodology Code: Liquid Chromatography
As microfluidic devices enter their second decade of development, commercial products based upon capillary electrophoresis separations in a microchip are available. The challenge now is to meet the early promise of on-chip sample processing, creating truly miniaturized and automated laboratory procedures for clinical chemistry, and to provide new tools for the burgeoning fields of functional genomics, and proteomics. Multiplexed systems, processing many samples simultaneously, offer the largest advantage of microfluidic systems.

This presentation will focus in part on devices designed to combine electrophoresis, protein digestion, preconcentration and electrospray-MS in 6- through 36-channel systems. Electrokinetically driven flow systems will be described for these purposes.

The other area where fluidic systems can contribute to biochemical sample analysis is in novel nano-porous materials for protein separation. Glancing angle deposition of nanoporous thin films can create highly porous materials of virtually any material or element that can be vacuum deposited. We have shown that these materials can be used for solid-matrix laser desorption ionization (SMALDI) of low mass molecules (70-3000 Da) with far less chemical background than seen in MALDI. They can also be used directly for ultra-thin layer chromatography (UTLC), allowing integration of two powerful technologies for biochemical analysis. Analysis of peptide and metabolite samples will be discussed in detail.
A new mode of the scanning electrochemical microscope (SECM) operation was developed that combines reagent delivery from the nanopipette with electron transfer at the conductive substrate and ion transfer across the liquid/liquid interface supported at the nanopipette tip. This approach offers potential advantages for measurements of heterogeneous electron transfer kinetics and reaction rate imaging by enabling straightforward separation of the contributions of surface topography and reactivity features to the tip current. It addresses some other long-standing technical issues, including sensitive probing of low signal sources (e.g., immobilized enzymes or catalyst particles) without diffusional broadening and the elimination of the elevated background signal in generation/collection-type experiments. The high spatial resolution attainable in electron transfer/ion transfer mode experiments and the absence of redox mediator species in the bulk solution are advantageous for studies of biological cells.

**Keywords:** Imaging

**Application Code:** General Interest

**Methodology Code:** Electrochemistry
We report the screening of electrocatalytic materials for using bipolar electrochemistry. Thus far, we have tested materials in an array-based format for the oxygen reduction reaction (ORR) and the hydrogen evolution reaction (HER). The materials are tested using bipolar electrodes (BPEs). A BPE is a conductive wire that drives both oxidations and reductions simultaneously. BPEs are typically powered by an externally applied electric field that results in variations in interfacial potential differences between the BPE and the electrolyte solution. BPE-based screening relies on the fact that each material requires a different overpotential needed to drive the reaction of interest, which can be compared to a reporting reaction that occurs on each BPE. The screening experiments are quick (~10 min) and require only very simple instrumentation (a DC power supply and a simple optical microscope). Because BPEs are operated without direct electrical connection to each electrode, an optical readout is necessary. We use the electrodissolution of thin metal microbands, such as Ag and CrOx, to report the properties of each candidate. The readout is simple: the more microbands that dissolve, the better the catalyst.

**Keywords:** Electrochemistry, High Throughput Chemical Analysis, Materials Characterization, Sensors

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

**Methodology Code:** Electrochemistry
Nicotinamide Adenine Dinucleotide (NAD+) is a cofactor for hundreds of dehydrogenase enzymes, many of which are currently being used in the development of electrochemical biosensors and biofuel cells.[1–4] Dehydrogenase enzymes will typically oxidize a substrate while concurrently reducing NAD+ to dihydronicotinamide adenine dinucleotide (NADH). Oxidation of NADH at an electrode surface is often used to electrochemically detect enzymatic turnover, and has the added benefit of regenerating the enzymatically active form of the cofactor. Oxidation of NADH, however, is difficult at traditional electrode surfaces due to the large activation energy or overpotential necessary to initiate the electrochemical reaction. Carbon Nanotubes have been shown to significantly reduce the required overpotential, thereby becoming an attractive electrode material for use with dehydrogenases.[5] Heteroatom-doped CNTs, such as boron-doped CNTs (B-CNTs), have been shown to further decrease the oxidation overpotential compared to nondoped CNTs.[6] Here, we report the use of nitrogen-doped CNTs (N-CNTs) as an effective electrocatalyst for NADH oxidation. More specifically, we investigate the electrochemical kinetics of NADH oxidation at N-CNTs, and their implications towards measuring the enzymatic turnover of dehydrogenase enzymes.


Keywords: Bioanalytical, Biosensors, Electrochemistry, Electrode Surfaces
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Ion-selective electrodes (ISEs) with fluororous anion-exchanger membranes [1] for the potentiometric detection of two environmental contaminants, perfluorooctanoate (PFO\(^{-}\)) and perfluorooctanesulfonate (PFOS\(^{-}\)), were developed. The sensors exhibit exceptionally high selectivities for PFO\(^{-}\) and PFOS\(^{-}\) over interferents that are commonly present in the environment. They also provide trace level detection limits for PFO\(^{-}\) and PFOS\(^{-}\), i.e., \(1.7 \times 10^{-10}\) M (0.070 ppb) and \(8.6 \times 10^{-10}\) M (0.43 ppb), respectively. These values are comparable with results obtained using well established techniques such as GC/MS, LC/MS, and LC/MS–MS, but the measurement with ISEs avoids lengthy sample preconcentration, can be performed in situ, and is less costly. Real life application of these electrodes was demonstrated by in-situ measurements of the adsorption of PFOS\(^{-}\) onto Ottawa sand and detection of PFOS\(^{-}\) in a background of water from Carnegie Lake. The results obtained are consistent with those from an earlier LC–MS study, validating the usefulness of these sensors for environmental studies. With a glass-free solid contact reference electrode, a field-deployable sensor system was fabricated. The sensor system was successfully applied for simultaneously detecting and monitoring of PFO\(^{-}\) and PFOS\(^{-}\) in contaminated soil, surface water and drinking water, demonstrating the fast, easy, sample pretreatment-free, selective and economical nature of PFO\(^{-}\) and PFOS\(^{-}\) detection with fluororous membrane ISEs.
A boron-doped diamond (BDD) electrode allows for a wider potential window for investigation of lanthanide trifluoromethanesulfonate (triflate) compounds. Electron transfer and mass transport kinetics are evaluated and compared for triflate complexed ytterbium (III), samarium (III), cerium (IV), and dysprosium (III).

The lanthanide elements can be difficult to distinguish from each other because they have extremely similar properties, including masses, ionic radii, oxidation states, and standard potentials. Present lanthanide detection and separation methods are tedious and time-consuming. Electrochemically, lanthanide analysis is also hindered by the magnitude of their standard potentials (in the range of -1.99 and -3.90 V vs NHE), which fall outside of the potential window of common electrode materials. For example, in aqueous solutions at platinum, the potential window is limited between +1.3 and -0.7 V vs NHE by solvent electroysis. Previously, researchers have resorted to mercury drop electrodes or chemically modified carbon paste electrodes to look at lanthanide compounds. BDD electrodes offer a safer, simpler alternative; the overpotentials for hydrogen and oxygen occur at very extreme potentials on BDD compared to other materials such as glassy carbon, platinum, and gold.

Solutions of 1.0 mM lanthanide (III) trifluoromethanesulfonate (Aldrich) and 0.10 M tetrabutyl ammonium bromide (Aldrich) in acetonitrile (Sigma) were prepared and degassed with N2(g). Cyclic voltammetry was performed using a 3 mm diameter boron-doped diamond working electrode (CCL Diamond), Ag/AgO quasi-reference electrode, and platinum mesh counter electrode.

Keywords: Analysis, Electrochemistry, Electrodes, Voltammetry
Application Code: General Interest
Methodology Code: Electrochemistry
Crystalline III-V semiconductor thin films are attractive components for energy conversion and storage technologies, electronic devices, and various sensing and transduction architectures. However, conventional production of these materials is energy intensive, rendering them incompatible with common heat-sensitive device fabrication schemes (i.e. CMOS, soft lithography, and flexible electronics). We have recently developed a near-ambient electrochemical approach that leverages direct electrodeposition of dissolved Group V oxides on metallurgically-active Group III metals to afford crystalline III-V thin films in water (Figure 1). A mechanistic understanding of the concerted chemical and physical processes relevant to the reactive metal electrode interface is critical for successful application of this technology. First, steady-state spectroelectrochemical and electroanalytical measurements will be shown that suggest a native In$_2$O$_3$ layer coating In($\text{i}$) film electrodes must be electrochemically removed prior to successful InAs film formation. Second, we will introduce an $\text{in-situ}$ electrochemical Raman spectroscopic technique which is used to investigate the real-time electrochemical As$_2$O$_3$(aq) reduction and interfacial In$^{(\text{i})}\text{As}^{(\text{i})}$ reaction dynamics (Figure 2). Key technical aspects of the method including cell design, temporal and spatial resolution, and strategies for mitigating Raman laser annealing artifacts will also be discussed. Real time potential-dependent Raman spectroscopic data will be shown that suggests the rate of InAs film growth is limited by the interfacial diffusion/reaction kinetics rather than the initial electrochemical reduction of As$_2$O$_3$(aq). Moreover, evidence will be provided that reveals formation of surplus As$^{(\text{i})}$ when the electrodeposition rate outpaces the interfacial reaction rate.

**Keywords:** Electrode Surfaces, Infrared and Raman, Semiconductor, Spectroelectrochemistry

**Application Code:** Materials Science

**Methodology Code:** Electrochemistry
Characterization of emerging solar materials is often complicated by the lack of established device fabrication procedures. Electrochemical contacts provide a means to characterize semiconductor materials without the need to fabricate solid state devices. We investigate GaAs thin films deposited via close-spaced vapor transport (CSVT) from powder precursors. Electrochemical measurements of CSVT GaAs facilitate rapid characterization of the dopant type and density, spectral response, J-E response, and minority carrier diffusion length $L_D$. The feedback from these measurements has spurred several improvements to our CSVT system, enabling deposition of GaAs thin films with controllable p- and n-type doping, internal quantum efficiency $\eta_{int} > 0.95$, and $L_D > 5 \mu m$. This work was supported by the U.S. Department of Energy SunShot Initiative (DE-EE0005957).

**Keywords:** Characterization, Electrode Surfaces, Energy, Semiconductor

**Application Code:** Materials Science

**Methodology Code:** Electrochemistry
Detecting bacterial pathogens during biosurveillance efforts and characterizing their origin is an important task for many forensic agencies. One of the biggest challenges is distinguishing between pathogens that occur naturally in the environment from those cultured in the laboratory for illicit activity. Because of the significant disparity in nutritional landscape between laboratory cultures and natural reservoirs, targeted biochemical profiling of intracellular lipids and trace metals are promising analytical strategies for differentiating these two pathogen sources.

For this study, \textit{Bacillus} [\textit{cereus}] spores were cultivated in both conventional media recipes and a simulated soil microcosm. After harvesting spores from both culture types, fatty acids were extracted from whole cells and derivatized into fatty acid methyl esters for GC-FID profiling. Results show that \textit{Bacillus} spores cultivated from a soil environment are depleted in branched-odd fatty acids, particularly 15:0 iso, compared to conventional laboratory cultures (~17% and ~34%, respectively). This decrease was accompanied by an enrichment of straight-chained fatty acids (16:0, 16:1w7c) in soil cultures (~35% compared to ~20% in laboratory samples). Additionally, compositional profiling was performed on \textit{Bacillus} spores with ICP-OES. Distinct elemental signatures were observed in each type of culture. Cd, Al, and Si were indicative of soil microcosms whereas organisms from conventional growth media exhibited higher abundances of Fe, Mg, and Mn.

Results from this study illustrate the utility of fatty acid and trace element profiling for forensic characterization of environmental and laboratory-grown pathogens. This analytical scheme has the potential to identify the source of unknown bacterial threat agents.

**Keywords:** Environmental/Soils, Forensic Chemistry, GC, ICP

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
Direct Analysis in Real Time-Mass Spectrometry (DART-MS) is a promising technique for rapid characterization of unidentified bacterial agents. DART-MS may be used in biodefense and forensic applications as it requires little to no sample preparation and analyte ionization occurs under ambient conditions. The goal of this study was to test whether DART-MS could be used to analyze Bacillus spore preparations and identifying the strain present as well as the culturing conditions. Two different strains of Bacillus cereus spores (T-strain and 14579) were grown with three different medium formulations, each containing a different complex carbon/nitrogen source (tryptone, peptone, and yeast extract). Whole spore suspensions from each culture were then analyzed directly with DART-MS.

Results show that MS profiles of spores consisted of complex assemblages of both fatty acids and amino acids. The range of lipid structures included branched-odd, branched-even, and straight-chained fatty acids. The relative ratios of fatty acid structures were indicative of both strain and the culturing medium recipe. 

**Bc14579** spores were enriched in 17:0 iso fatty acids whereas **BcT** spores were enriched in 13:0 iso and 15:0 iso fatty acids. DART-MS profiles of spores of either strain grown in tryptone formulations showed a higher proportion of 15:0 iso fatty acids whereas peptone cultures showed an increase in 14:0 iso and 16:0 iso fatty acids. The amino acid profiles were equally informative for determining the primary nutrient source. These results suggest that DART-MS can be a powerful tool for taxonomic characterization of unidentified spores as well as determination of forensically relevant aspects of the culturing procedure.
Abstract Text

The need for a bioagent analyzer to protect the military abroad and US citizens at home was emphasized by Secretary of State Hillary Rodham Clinton at the December 2011 Biological Weapons Convention held in Geneva, who stated “Unfortunately, the ability of terrorists to develop and use these weapons is growing”; and was once again demonstrated in May 2013 by the mailing of Ricin to President Barack Obama, New York City Mayor Michael Bloomberg and, Director of Mayors against Illegal Guns Mike Glaze. Despite the substantial effort to develop bioagent analyzers, current analyzers are either too slow (e.g. PCR, 1 measurement/hour), have high false-alarm rates (e.g. immunoassays), lack sensitivity (e.g. Raman), are not field-usable (e.g. GC-MS), or cannot be multiplexed to identify multiple species (e.g. PCR). Consequently, there is a critical need for a field-usable analyzer that can detect a broad range of bioagents (in air or water) at exceptionally low concentrations (e.g. 10e4 B. anthracis spores per liter water), and at relatively fast speed (minutes) to minimize casualties. To meet this need we have been developing a sample system that selectively binds specific bioagents and produces surface-enhanced Raman spectra (SERS). Here we present discriminate detection of bioagents (e.g. B. anthracis spores, Ricin toxin, and F. tularensis) in 15 minutes measured at the US Army’s Edgewood Chemical Biological Center.

Keywords: Bioanalytical, Enzyme Assays, Lab-on-a-Chip/Microfluidics, Surface Enhanced Raman
Application Code: Homeland Security/Forensics
Methodology Code: Vibrational Spectroscopy
The goal of this study was to investigate surface associated fatty acids as a forensic signature for the attribution of [i]Bacillus[/i] spores. During laboratory culturing, fatty acids are released from vegetative cells into the growth medium and become adsorbed to surfaces of intact spores. The types of fatty acid structures and their relative abundance can potentially be used to identify the species/strain of [i]Bacillus[/i] and determine key aspects of the growth medium used for spore cultivation. To test the forensic utility of surface-associated fatty acids, three different types of [i]Bacillus[/i] organisms were cultured: [i]Bacillus cereus[/i] T-strain, [i]Bacillus cereus[/i] 14579, and [i]Bacillus subtilis.[/i] In addition, each organism was cultured in three medium formulations, each with a different complex nutrient source (tryptone, peptone, and yeast extract). Fatty acids were extracted from the cell surface with acetone and then derivatized into fatty acid methyl esters for GC-FID profiling.

Results showed that the types and relative abundance of different fatty acid structures on the spore surface could be used to determine both the taxonomy and the primary nutrient source for an unknown [i]Bacillus[/i] sample. Specifically, surfaces of [i]B. subtilis[/i] spores were enriched in 15:0anteiso compared to [i]B. cereus[/i], and each strain could be differentiated by the abundance of 15:0iso and 17:0iso fatty acids. Distinct variation in 17:0anteiso, 14:0iso, and 17:1iso5c markers indicated growth in each of the three nutrient sources. These methods could be inserted into existing operational workflows and provide novel data streams during a forensic investigation while preserving the sample for further analyses.

Keywords: Biological Samples, Forensics, Gas Chromatography, Identification
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography
Cluster Analysis of Smokeless Powders and Classification by Discriminant Analysis

The purpose of this research is to determine if statistically valid classes can be identified beyond single- and double-base designations for smokeless powders. A larger number of smokeless powder classifications will aid in the forensic analysis of these materials. Smokeless powders are low explosive propellants in military and civilian ammunition, and are commonly used in improvised explosive devices (IEDs). The National Center for Forensic Science in collaboration with the Explosives Committee of the Scientific Working Group for Fire and Explosions (SWGFEX) has developed a database comprised of a compilation of analytical data for commercially available smokeless reloading powders. For this study, GC-MS data for 726 powders was used to generate their total ion spectra (TIS). The TIS was calculated by summing the intensities for each nominal mass across the entire chromatographic profile, and normalizing the summed intensities to the base peak. The TIS was used as input data to perform agglomerative hierarchical cluster (AHC) analysis. AHC analysis was used for the identification of classes within the smokeless powders TIS. Correlation distance and average linkage were used to perform the cluster analysis and six distinct classes were identified within the dataset. Linear discriminant analysis (LDA) was tested by jackknife cross validation to estimate correct classification rates for assigning samples to the six classes. The smokeless powder cross validation samples classified into their respective classes with a correct classification rate of 98%.

This research was conducted at the National Center for Forensic Science, a State of Florida Type II research center.

Keywords: Chemometrics, Forensics, Gas Chromatography/Mass Spectrometry, GC-MS
Application Code: Homeland Security/Forensics
Methodology Code: Chemometrics
The use of homemade explosives poses a growing threat in the United States. Recently, explosives in which the oxidant is Trichloroisocyanuric acid (TCCA), the primary component in swimming pool chlorinator tablets, and the accelerant is ethylene glycol derived from automotive brake fluid have gained attention from the forensic community. Despite their potential to be used for illicit activities, few forensic signatures exist for the attribution of chlorinator bombs. Therefore, the goal of this research was to analyze trace chemical profiles of different manufacturers of chlorinator tablets and automotive brake fluid using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), X-ray Diffraction (XRD), and Gas Chromatography-Mass Spectrometry (GC-MS) respectively. Chemical signature profiles obtained for precursor components were compared to signatures obtained from post-blast residues.

Results showed that different brands of brake fluid exhibited characteristic GC-MS profiles. Various glycols and glycol ethers were uniquely present in the profiles of the different brake fluids. Many of the compounds that were common to all three brake fluid brands showed distinct variation in their relative abundance across profiles. These observed differences in the precursor materials were also observed in the GC-MS profiles of the post-blast explosive residues. ICP-OES analysis revealed that among different brands of TCCA pool chlorine there is significant variation in the concentration of copper, aluminum, sodium, and magnesium which is consistent in pre and post-blast materials. Thus this research poses promising forensic trace signature profiles for both TCCA swimming pool chlorine and automotive brake fluid that are used in homemade chlorinator explosives.

Keywords: Forensic Chemistry, GC-MS, ICP-MS, Trace Analysis
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography/Mass Spectrometry
Emplaced explosives (land mines, etc.) are a potential danger to a war fighter and the need to detect and characterize any potential threat encountered in the field is essential. To address these threats, we have developed STARR (Shortwave-infrared Targeted Agile Raman Robot), a robot mounted sensor capable of identification and confirmation of potential threats. The system utilizes shortwave infrared (SWIR) spectroscopy for the identification and automated targeting of potential threats, combined with a visible short-range standoff Raman hyperspectral imaging (HSI) system for material confirmation. The entire system is mounted onto a Talon UGV (Unmanned Ground Vehicle), allowing the sensor an increased area search rate and reducing the risk of harm to the operator. The Raman HSI system utilizes a fiber array spectral translator (FAST) for the acquisition of high quality Raman chemical images, allowing for increased sensitivity and improved specificity. This presentation will include a discussion of the SWIR and Raman sensors, show how the SWIR directed targeting functions, and give initial results for various operating scenarios.

Keywords: Detection, Infrared and Raman, Instrumentation, Molecular Spectroscopy
Application Code: Homeland Security/Forensics
Methodology Code: Molecular Spectroscopy
The threat of terrorism or criminal bombings has become a serious problem worldwide. Most of commercial ETDs require human operations for swab-sampling from the surfaces of the inspection objects, which makes 100% inspection very costly. Therefore, an auto-sampling ETD would dramatically reduce the need for human operations, which can be applied to all baggage or human screening at a much lower cost. To analyze various explosives including TNT, PETN, and RDX, we developed a novel particle auto-sampler using a cyclone-type particle concentrator [1]. This auto-sampler can collect and concentrate the explosive particles attached on the objects effectively and rapidly. The heating unit that is placed in the bottom of the cyclone concentrator vaporized the particles into the vapor instantly. Mass spectrometer real-time detects the presence of explosives contained in the vapor with its high selectivity. This configuration enriched the concentration of explosives concentration by 50-1000 times compared to that without the concentrator. We’ll report 2 types of inspection systems that were deployed with the auto-sampling ETD. 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. [1] Y. Hashimoto et al, “Development of Explosives Trace Detection System using Cyclone-type Particle Concentrator”, ASMS2012, May, 2012, Vancouver. Canada.
The objective of this research is to develop an analytical strategy to determine emerging compounds of concern. Wastewater samples obtained from the Pennsylvania State University wastewater treatment facility (WWTF) will be used as a control facility to refine analytical methodology. Rather than beginning with a target compound approach, a discovery analysis approach was chosen to try and determine as many compounds as possible prior to any compound list restriction. The difficulty in this approach can be the resulting complexity of the analysis. Both Comprehensive Gas Chromatography coupled with Time-Of-Flight Mass Spectrometry (GC x GC-TOFMS) analysis and Ultrahigh Performance Liquid Chromatography coupled with Time-Of-Flight Mass Spectrometry (UHPLC-TOFMS) analysis were utilized for their inherent ability to characterize these potentially complex samples more successfully compared to other possible techniques. The ultimate goal is to determine emerging contaminants and define temporal and spatial characteristics of occurrence. Equally challenging is the need to develop and define what is to be considered “normal” so that this background can be subtracted from subsequent samples in order to develop an approach that is capable of determining when an “outlier” is detected. This presentation will address how these large data sets can be reduced to allow for more easy determination of what are outliers.

Multiple four-liter samples were gathered from stages throughout the Penn State WWTF. Samples were prepared following USEPA method 3510c, a liquid-liquid extraction. Also, soil samples were collected from the living filter (final discharge for the Penn State WWTF effluent) and extracted following USEPA 3550c, a sonification method, in order to help define environmental fate of outliers found in the treated waters. Sample extracts were introduced to the analytical systems, to identify and quantify the detected compounds.

Keywords: Environmental, Environmental/Soils, Environmental/Waste/Sludge, Environmental/Water
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Cell-based sensing is a valuable approach for the detection of both known and unknown environmental toxicants. Monitoring the response of cultured cells after exposure to an environmental water sample reveals the sample cytotoxicity. We have developed a portable cell-based unit for on-site detection of chemical hazards in environmental waters. Herein, two innovative tools integrated in the portable detection unit are presented. The tools are designed with simple working principles and uncomplicated fabrication processes. The first tool is a fully automated device that adjusts the samples’ osmolality and pH in-line. In cell-based sensing, adjustment of the samples’ properties before introducing them into the cell culture is essential. Our novel osmosis-based technique exploited in this device avoids dilution of the toxicants and denaturation of the ingredients. The second tool is an enzyme-based microreactor cartridge for simultaneous amperometric measurement of glucose and lactate concentrations in a few microliter culture medium extracted from the cell culture. Monitoring the metabolism of glucose and lactate is a cytotoxicity criterion that can be used for almost all cell types. The microreactor cartridge designed is a microfluidic device that monitors the cells’ metabolism outside the culture; thus, avoids contaminating the cells. Furthermore, a protocol for preparation of the culture-medium samples before introducing them into the cartridge is proposed. This protocol prevents the interferences caused by the toxicants that inhibit the enzymes functionality or interfere with the amperometric detection. This work was supported by the Nano-Tera Livesense project funded by the Swiss National Science Foundation (SNSF).
Nutrients (i.e., nitrogen and phosphorus) are a natural part of the aquatic ecosystems that help support the growth of algae and aquatic plants, which provide shelter and food for fish and other organisms. However, excess nitrogen and phosphorus in the waters, such as from anthropogenic sources, can cause serious environmental and health issues. Excessive nutrients in the water can create algal blooms, which can decrease the oxygen in the water that are needed by fish and other aquatic life to survive. Some algal blooms are also harmful to humans because they produce elevated toxins and bacterial growth that can cause illnesses when coming in contact with the water, consuming tainted fish, or drinking contaminated water. Because a national numeric criteria doesn’t exist, the EPA requested that each state develop criteria for their water bodies. Simultaneous nitrogen and phosphorus determinations are typically accomplished by Kjeldahl digestion methods. However, this procedure suffers from several drawbacks; such as increased safety risks, increased cost to dispose of mercury, and by providing values for organic nitrogen and ammonium only (excludes inorganic nitrite and nitrate). Alkaline persulfate digestion for simultaneous total nitrogen and phosphorus determinations is a more sensitive, accurate, and less toxic alternative to Kjeldahl digestion. In this report, alkaline persulfate digestion was used to convert nitrogen to nitrate and phosphorus to phosphate followed by determination using anion exchange chromatography with suppressed conductivity detection. To initially evaluate the method, several inorganic and organic nitrogen and phosphorus-containing compounds were digested and the recoveries were determined. For the nitrogen-containing compounds, the recoveries ranged from 93-100% and from 85-99% for the phosphorus-containing compounds. The linearity, sensitivity, and recovery of nitrate and phosphate in environmental waters will be discussed.
Environmental Analysis of Non-Metals in Water (Half Session)

Determination of UV Filter and Biocide Compounds in Surface Water Samples Using High Throughput Solid Phase Microextraction System Coupled with Liquid Chromatography–Tandem Mass Spectrometry

In this study, high throughput solid phase microextraction method was developed for analysis of UV filters and biocide compounds in surface water samples. Liquid chromatography and tandem mass spectrometry methods were optimized and applied for separation and detection of the compounds. As a sample preparation method, thin film solid phase microextraction (TF-SPME), a rapid, environmentally friendly, and a sensitive analytical technique which isolates and pre-concentrates trace organic pollutants from environmental samples in a single step was applied. The evaluation of four different extraction phases with various desorption solvents for extraction of target compounds showed that hydrophilic-lipophilic balance particles (HLB) coated SPME blades and methanol are the best in terms of percent recoveries and carryover on the blades. SPME method was optimized in terms of preconditioning, extraction and desorption times. The optimum extraction and desorption times were determined as 60 minutes, both with agitation. A flow-through standard water generation system was used in all method development steps as a reliable and reproducible technique for preparation of standard aqueous solution. Wide linear dynamic ranges with developed method were obtained for each compound, which enables to use the developed method for a wide range of concentrations. Application of the method was demonstrated using environmental water samples from wastewater-dominated reaches of the Grand River (adjacent Waterloo, ON).

Keywords: Environmental/Water, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry, SPME
Application Code: Environmental
Methodology Code: Liquid Chromatography
High Resolution NMR Spectroscopy offers unique screening capabilities for food quality and safety by combining non-targeted and targeted screening in one push button analysis taking 15-20 minutes. The objective is to demonstrate, that due to its extreme reproducibility and transferability NMR can detect smallest changes in concentrations of many components in a mixture, which is best monitored by statistical evaluation however also delivers reliable quantification results. The methodology typically uses a 400 MHz high resolution instrument under full automation after minimized sample preparation. One analysis operation delivers a multitude of results, which are automatically summarized. The method has been proven on fruit juices, where so far unknown frauds could be detected, be it addition of sugars and amino acids to fake 100% fruit content, mixing of different varieties or wrong product labelling like geographical origin and direct juice versus rediluted concentrate as well as on wine. The methodology developed is now transferred to wine quality control. The advantage of NMR is its reproducibility and transferability from instrument to instrument. Such it is possible after setting up standard operation procedures to perform targeted analysis (identification/quantification) on a multitude of compounds and non-targeted analysis to generate information like grape variety, origin, vintage year and unexpected/unknown deviations. This technology has the advantage that NMR is completely quantitative. Concentration calibration only has to be done once for all compounds. Since NMR is so reproducible (based on strict SOP’s), it is also transferable between different instruments of the same field strength and different laboratories. Having established the statistical and quantification methodology, it is scheduled to expose the technology described for fruit juices and wines to other food material by exchanging the underlying spectral database to honey and edible oil.

Keywords: Analysis, Beverage, NMR, Quality Control
Application Code: Quality/QA/QC
Methodology Code: Magnetic Resonance
We have been developing sugar-based deep eutectic solvents (DES) using biochemical sugars such as glucose (GL), fructose (FR) and sucrose (SU) combined, in various molar ratios, with small acids namely Malic acid (MA), Maleic acid (ME) and Aconitic acid (AA), Citric acid (CA) and Choline Chloride (CC) and water (H). The objectives are to characterize these DES and determine their applications. FR:CC:H (1:1:1) and GL:CC:H (1:1:1) underwent facile melting without application of heat. Generally, the other DES required temperatures of 75oC and 200 rpm for 3 hours to prepare whilst some remained solid. Water content analyses using the Karl-Fisher Titrator gave a range of 1.39% (MA:CC, 1:1 with a mass of 0.034 g) to 6.81% (GL:CC:H, 1:1:1 with mass of 0.0186 g). FT-IR analyses have been done on all individual samples and the DES to identify unique peaks associated with the DES. We continue to determine the melting points, TGA/DSC, viscosity, octanol/water partition coefficient, polarizability, membrane diffusion, toxicity, and enzyme activity of the DES.

Keywords: Analysis, FTIR, NMR
Application Code: General Interest
Methodology Code: Physical Measurements
Microwave-assisted sample preparation has been the gold standard for sample digestion trace analysis for the past two decades. Although there have been a number of studies illustrating advancements in microwave digestion techniques, conventional microwave designs have limited temperatures, pressures and the types of matrices that can be processed effectively.

A shift in microwave design to a single reaction chamber (SRC) technology has allowed significant advancements in how samples are digested. This change enables a number of advantages over traditional approaches including: 1) mixed batch and mixed acid chemistry executed simultaneously, 2) temperatures of 300°C and 199 bar and 3) the flexibility of utilizing disposable glass, Teflon or quartz vials in multi-position rack. To go along with increased capability, SRC technology has broadened the sample type and increased the quantity that can be digested, allowing for a more complete solution in metals preparation.

Organic petro and chemical oils, plastics and polymers, along with specific types of consumer products are sample matrices with high organic content and provide significant challenges in obtaining high quality ICP-OES/MS data due to high pressures generated in the organic decomposition and the high residual carbon content remaining in an incomplete digestion. Using SRC technology a study of the critical parameters and best practices necessary for complete digestion across a wide range of complex organic samples will be presented for trace metals analysis with ICP-MS data.

Keywords: ICP-MS, Metals, Microwave, Sample Preparation
Application Code: General Interest
Methodology Code: Sampling and Sample Preparation
Identity testing of raw materials for potency and verification of label claim data in nutraceutical formulations are requirements of dietary supplement cGMPs. Section 21CFR111.320 of cGMPs for Dietary Supplements requires you to “identify and use an appropriate scientifically valid method for each established specification for which testing or examination is required to determine whether the specification is met”. The FDA does not elaborate on what is considered a scientifically valid method in the cGMPs.

There is much debate over what validation should entail, but at the most basic level a scientifically valid method should meet minimum linearity, precision, sensitivity and range requirements. These requirements are outlined in an FDA laboratory document, ORA LABORATORY PROCEDURE Food and Drug Administration, ORA-LAB.5.4.5. This laboratory guidance document defines minimal performance attributes for selected methods of analysis.

Manufacturers and contract testing labs alike are looking for accurate and scientifically valid methods that are suitable for use with different formulations. The complex nature of nutraceuticals and botanicals often requires long analysis and difficult sample cleanup steps to resolve matrix interferences. In this talk, we will describe the development and validation process for three different dietary supplements: Ginger, Green Coffee, and Stevia. Each material required a different type of validation: ginger was a limit test, green coffee determined raw material potency, and stevia was an example of a final QC release test. The requirements of the validation were slightly different for each type of test.
Multidrug membrane transporters (efflux pumps) in both prokaryotes and eukaryotes are responsible for ineffective treatment of a wide variety of diseases, including infections and cancer, underscoring the importance of better understanding their structures and functions for design of effective therapies. We have developed far-field photostable-optical-nanoscopy (PHOTON), which includes photostable single nanoparticle imaging probes, single nanoparticle plasmonic microscopy and spectroscopy. We have demonstrated that PHOTON can be used to characterize the efflux function of single membrane transporters in single live cells in real-time at single-molecule (SM) and nanometer (nm) resolutions for better understanding of multidrug resistance (MDR). The updated results and applications will be discussed in this presentation.

The work is supported in part by NSF (CBET 0507036) and NIH (R01 GM0764401; 3R01 GM076440-04S1).
Bacteria are known to naturally aggregate and adhere to surfaces forming biofilms. Bacteria within biofilms live in a complex microbial community that exhibits primitive homeostasis, a circulatory system, and metabolic cooperativity.[1] Corresponding to differences in phenotype, biofilm bacteria show different gene expression profiles than planktonic bacteria. Biofilms can convey either detrimental or beneficial effects, depending on the setting. For example, they are beneficial in biotechnology applications, such as wastewater treatment.[2] On the other hand, biofilms can be pathogenic by exhibiting increased resistance to antimicrobial agents and the immune system of host organisms.[3] Characterizing and spatially mapping the chemical and molecular composition of bacterial biofilms provides valuable insight into the mechanisms of formation, the function of chemical constituents and how they may be managed. In this work, Raman spectroscopy and imaging have been used for the chemical analysis of two different bacterial strains, Pseudomonas sp. GM41 and Pantoea sp. YR343, allowing us to differentiate the two strains based on their vibrational signatures, study the process of biofilm formation and visualize the distribution of different biomolecules which include various excreted metabolites that are found within the biofilm matrix.

The authors acknowledge funding from the Department of Energy Office of Science (BER) through grant DE-SC0006642 and the Genomic Science Program, Plant-Microbe Interfaces Project (ORNL).

High fidelity hyperspectral imaging can be performed using the point mapping, line-scan imaging, or wide-field (global) imaging modalities. Low light applications, however, benefit from spatial multiplexing approaches in which the light corresponding to individual pixel locations in the sample plane is combined so that the intensity from many pixel regions on the sample are recorded simultaneously using a single channel detector. A series of measurements are required, one for each combination of pixel elements. In applications like Raman hyperspectral imaging, spatial multiplexing enables lower laser power densities to be employed, thereby reducing the risk of sample damage. In the work presented here, a digital micro-mirror device (DMD) microscope has been constructed for Raman and Brillouin imaging. In our implementation, simplex encoding matrices govern the combination of pixels used for each reading and Gaussian elimination is used to recover the individual image pixel intensities. A challenge for multiplexed imaging of inelastically scattered light is reducing the effect of photo-bleaching. Because the overall intensity of the signal decreases in time in the presence of fluorescence quenching, it is difficult to recover the individual pixel intensities accurately. As part of this work, we have developed a novel method for correcting temporally dependent fluctuations in the measured light intensity. The design of the DMD imaging system and a theoretical description of the intensity correction algorithm are described.

**Keywords:** Bioanalytical, Imaging, Instrumentation, Raman

**Application Code:** Bioanalytical

**Methodology Code:** Surface Analysis/Imaging
Functionally graded surfaces have found applications in the chromatographic separations, microfluidic devices and in biosensors. Various substrates have been used for preparation of gradient surfaces and have found different applications such as energy free transportation of fluids and protein and cell adhesion and growth. In our research we have focused on preparation of chemical gradients on cellulose paper, specifically water color paper using amine, phenyl and thiol alkoxysilanes. Radial and linear gradients were prepared using controlled rate infusion method. The silane sols were prepared such that its silanol groups were hydrolyzed and could react with the hydroxyl groups of cellulose as well as to each other. Amine and thiol gradients were characterized by uv-visible spectroscopy using ninhydrin and ellman’s reagents, respectively while diffuse reflectance spectroscopy was used for characterizing phenyl gradient. Contact angle measurements further confirmed the presence of gradients. SEM images were also obtained to see the changes in the paper morphology due to the infiltration of the different silanes. The stability of the concentration gradient on cellulose surface was analyzed by exposing the gradient cellulose surface to various stress conditions like heat, acid, base etc. Apart from that the stability of these gradients was also evaluated in polar and non-polar solvents by exposing them for different time periods. In this presentation the details about the preparation of radial and linear gradient on cellulose paper, their characterization and their stability upon exposure to various stress conditions will be presented.

Keywords: Materials Science, Spectroscopy, UV-VIS Absorbance/Luminescence
Application Code: Materials Science
Methodology Code: Surface Analysis/Imaging
For quantification of biotherapeutics LC-MS/MS has advantages in short development times, high accuracy and precision, multiplexing, no cross-reactivity, and facile distinction between closely related analogs. Intact insulins are difficult to analyze by LC-MS/MS, as MS sensitivity is low and insulin and its analogs suffer from non-specific binding and poor solubility, making LC and sample preparation method development difficult. A few LC-MS/MS methods exist. Most involve time-consuming and laborious immunoaffinity purification or nano-flow LC. This work provides a simple method for the simultaneous quantification of intact human insulin and 5 critical analogs in human plasma, achieving LODs of 50-100 pg/mL for each. Average accuracy and precision for standard curve and QC’s samples were 90-95%. Matrix factors for all analogs were calculated in 6 sources of human plasma and CV’s were <15% in all cases, supporting the selectivity of the method.

A specific challenge when quantifying insulins is the ability to distinguish between human insulin and insulin Lispro, as they differ by a reversal in the position of 2 amino acids. Only a single low molecular weight fragment differentiates the two, making selective sample prep and chromatography critical. This methodology takes advantage of mixed-mode SPE to eliminate interferences and a novel ultra-high efficiency charged surface column which produces narrow peak widths and improved mass transfer to facilitate high sensitivity quantification. Selectivity studies show that the presence of high levels of human insulin (i.e., type II diabetes), does not interfere with quantification of lispro or any of the other analogs.

Keywords: Bioanalytical, Biological Samples, Mass Spectrometry, Peptides
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Trace Level Neuropeptide Detection by Capillary LC-MS

Microdialysis of neuropeptides in the brain provides direct correlation between neuropeptide's in vivo concentration and brain function. A highly sensitive method is needed for neuropeptide detection in microdialysate due to the low (pM) concentrations and small samples generated. In the past two decades, capillary liquid chromatography (CLC) coupled to electrospray ionization mass spectrometry (ESI-MS) has become a powerful tool for analyzing neuropeptides in in vivo microdialysate. This technique was reported to have low attomole limit of detection (LOD) for certain neuropeptides including methionine enkephalin, leucine enkephalin, oxytocin and arg-vasopressin. Although successful, measuring bigger neuropeptides and neuroproteins with CLC-ESI-MS still remains challenging because the multiple charge distribution of bigger neuropeptides and neuroproteins in ESI-MS decreases MS signal intensity. In addition, sample loss caused by peptide/protein adsorption in the LC system prior to injection further reduces sensitivity. To address this issue, we have tried either adding organic additives to the sample to prevent adsorption, or enzymatically digesting big neuropeptides/proteins into smaller signature peptides which could be easily detected by ESI-MS. The organic additive method has been proven to enable detection for several neuropeptides including orexin A and B, galanin and Cholecystokinin-4 not previously detectable in dialysate. By selecting the optimal organic additive content, standard injection of 1 pM (8 amol) peptide was readily detected. We also performed enzymatic digestion on the neuroprotein brain derived neurotrophic factor (BDNF), and found that while trypsin was unable to digest this protein at nM range, chymotrypsin showed much better proteolytic activity. By monitoring a chymotryptic peptide peak, we were able to lower the LOD from high nM range for intact protein down to 10 pM (500 amol).

Keywords: Bioanalytical, Capillary LC, Liquid Chromatography/Mass Spectroscopy, Peptides

Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
One of the most important goals in metabolomics studies is introduction of a method for analysis of a large number of metabolites from the living system. Metabolomics data provides complementary information to proteomics, genomics, and transcriptomics, which makes tracking the dynamic reactions in living system easy. In bacterial metabolomics, quantitative and comprehensive metabolic map of cellular metabolites have a challenging workflow. Sampling and sample preparation perform a seriously important role in untargeted analysis as it affects the composition of the analyzed metabolome. In current work, we focused on development of different SPME coating chemistries to provide simultaneous extraction of a wide range of both hydrophobic and hydrophilic cellular metabolites. Also, two different LC-MS methods were used for reliable measurement of extracted metabolites. Data analysis showed that using newly developed 96-blade PS-DVB-WAX: HLB (50:50) coupled to developed UPLC (PFP)-LC/MS provides the widest metabolomics coverage for the understudied system. Changes in metabolomics footprinting of Escherichia coli under different environmental conditions (addition of cinnamaldehyde as an antibacterial agent above minimum inhibitory concentration and control conditions) were successfully investigated using SIMCA P+ software. As the maximum number of metabolites was detected in the exponential phase of bacterial growth curve, the study was done in this stage of growth of bacteria. More than 500 cellular metabolites were separated and detected in different chemical groups: amino acids, amino acids derivatives and peptides, nucleoside, carbohydrates, vitamins, and lipids. Antibacterial agents affects the cell growth and novel potential biomarkers were investigated via comparison of the bacterial cell under two different environmental conditions.

Abstract Text

One of the most important goals in metabolomics studies is introduction of a method for analysis of a large number of metabolites from the living system. Metabolomics data provides complementary information to proteomics, genomics, and transcriptomics, which makes tracking the dynamic reactions in living system easy. In bacterial metabolomics, quantitative and comprehensive metabolic map of cellular metabolites have a challenging workflow. Sampling and sample preparation perform a seriously important role in untargeted analysis as it affects the composition of the analyzed metabolome. In current work, we focused on development of different SPME coating chemistries to provide simultaneous extraction of a wide range of both hydrophobic and hydrophilic cellular metabolites. Also, two different LC-MS methods were used for reliable measurement of extracted metabolites. Data analysis showed that using newly developed 96-blade PS-DVB-WAX: HLB (50:50) coupled to developed UPLC (PFP)-LC/MS provides the widest metabolomics coverage for the understudied system. Changes in metabolomics footprinting of Escherichia coli under different environmental conditions (addition of cinnamaldehyde as an antibacterial agent above minimum inhibitory concentration and control conditions) were successfully investigated using SIMCA P+ software. As the maximum number of metabolites was detected in the exponential phase of bacterial growth curve, the study was done in this stage of growth of bacteria. More than 500 cellular metabolites were separated and detected in different chemical groups: amino acids, amino acids derivatives and peptides, nucleoside, carbohydrates, vitamins, and lipids. Antibacterial agents affects the cell growth and novel potential biomarkers were investigated via comparison of the bacterial cell under two different environmental conditions.

Keywords: Bioanalytical, Food Science, HPLC, SPME
Application Code: High-Throughput Chemical Analysis
Methodology Code: Liquid Chromatography/Mass Spectrometry
Top-down proteomics has brought increasingly attention; however, the separations of intact proteins are challenging and not satisfying. Here we report a MS compatible nano reversed phase column with pulled tip, which is packed with 470 nm silica nonporous particles. Model proteins: ribonuclease A, ubiquitin, superoxide dismutase, trypsin inhibitor and carbonic anhydrase were well separated in a 10 min gradient at room temperature with peak widths (4) ranging from 3.1 s to 5.6 s. Slower gradient gives better resolution and higher peak capacity. The retention times are reproducible within 0.3% RSD. These results indicate that the capillary packed with sub-0.5 μm silica particles with pulled tip is a good candidate for top-down proteomics.

This work is supported by grant NIH R01 GM1011464.

Keywords: Capillary LC, Liquid Chromatography, Protein, Proteomics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Liquid Chromatography/Mass Spectrometry
Oxidative stress, a major component of diabetic complications, causes reduced thiols to form disulfide bonds instigating an equilibrium shift in further downstream metabolic pathways. The goal of this work is to develop a method for the extraction, identification and separation of thiol metabolites. Reactive fluorous tags are implemented to extract thiol metabolites from the metabolome. This method uses fluorous interactions and then separates the fluorous thiols on a HILIC column according to the interactions from the thiol metabolites. Thiol standards were reacted with a fluorous maleimide at a ratio of 100:1 in an 80% acetonitrile buffer containing 5mM ammonium formate at pH8. The reaction time and temperature were optimized. The mixture of thiol metabolites was then separated using a cyano HILIC column with a linear gradient from 90% to 70% acetonitrile. Tagged thiols were detected using a mass spectrometer with an electrospray ionization probe run in positive mode. Signal intensity from fluorous-tagged thiols was improved over the untagged thiols indicating that this is a quick method that can be used to compare levels of reduced thiols in diabetic and normal cells. This method will further elucidate the impact of oxidative stress on thiol metabolism and therefore possible therapeutic targets for the treatment of diabetic complications.
Improving sensitivity using small sample volumes is particularly important in the bioanalysis of peptides and proteins. Historically this work was done by various ligand binding assays (LBAs) which are characterized by very high sensitivity (low fmol/mL or lower) and utilize only a small volume of sample, several µL or less. LBAs suffer some significant disadvantages though, including lack of standardization, poor linear dynamic range, variability and availability of reagents, and long development times. These disadvantages led to the desire to use LC/MS in this research area. However, obtaining equivalent sensitivity and sample usage to LBAs has been challenging. Nano and microscale LC/MS offers significant sensitivity advantages in supporting microsampling, biomarker discovery, and bioanalysis of biomolecules. In this work, prototype 150 µm i.d., 50 mm microfluidic devices packed with charged-surface 1.7µm (both fully porous and solid core particles) were evaluated for the high sensitivity analysis of the peptide teriparatide. Teriparatide is a 4118 MW fragment of human parathyroid hormone, used to treat osteoporosis. A multi-step sample preparation method using protein precipitation followed by solid phase extraction selectively separates teriparatide from closely related endogenous peptides and reduces interferences. Samples were concentrated 6X, without evaporation, minimizing the risk of adsorptive losses. Samples were run with injection volumes from 1 to 5 µL (approximately 200 to 1000 µL on a 2.1 mm i.d. column) under linear gradient conditions after trap and back elute on a 300 µm x 25 mm C18 trapping column. The resultant LC/MS method is equivalent to LBAs without its disadvantages.

Keywords: Bioanalytical, Biological Samples, Liquid Chromatography/Mass Spectroscopy, Peptides
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
On the floor of Pittcon 2009, along with a mass spectrometry firm, and observed by scientists from the University of Chicago, the University of Illinois, we demonstrated that droplets could be inductively energized and quantitatively shot into a TOF yielding ESI mass spectra. Specifically, we inductively shot single droplets of ppb chlorpromazine solutions into an ESI ToF, and we observed a mass spectrum identical to that of chlorpromazine acquired via traditional conductive ESI. That was a first, 100% ESI MS sample input via electric induction.

Recently in Analytical Chemistry, we published newer IBF data for Lanthanide element series chelates of octyl,phenyl,(N,N-diisobutylcarbamoyl) methylphosphine oxide (CMPO) using a programmable IBF source. We again showed the ability to launch 100% of droplets into a TOF MS, but now in as fast as millisecond time frames. We measured and observed excellent isotope ratios for low femtogram quantities of these analytes. In fact, in two hours CMPO chelates of the entire Lanthanide series of elements were determined in both positive and negative ion modes, running at ca. 0.4 Hz and slower. We've subsequently wondered if the entire Lanthanide and Actinide series could be so analyzed in one 50 nL drop.

More recently, IBF has been shown to yield reproducible ESI patterns for droplets of mixtures shot directly into ESI/MS's including: coffee; urine; alkali and transition metals; products, drugs and cellular liquids identifying components therein. We report here for the first time, IBF work on the LC/ESI/MS of oligonucleotides and vitamin D. In all cases, the IBF mass spectra observed for even polycharged analytes, are shown to be identical to conductive ESI mass spectra with low femtogram sensitivites. Nevertheless, given the 100% introduction efficiency, we seek routine attogram detection levels. We report on our progress, as we address the physics of spraying, electric induction and IBF analysis logistics.
N-glycan profiling often achieved by liquid chromatogram separation interfaced with mass spectrometry. Numerous separation techniques, such as hydrophilic-interaction liquid chromatography (HILIC), porous graphitized carbon (PGC) chromatography and reversed-phase chromatography, have been applied to separate N-glycans. PGC column is known for the strong isocratic separation ability of native N-glycans, while the C18 column is a powerful tool to separate permethylated N-glycans with increased sensitivity. However, the direct comparison between the two different columns is still lacking. In this study, equal amount of permethylated N-glycans and native N-glycans were analyzed on both C18 and PGC columns, respectively. The results suggest that PGC column is able to separate more isomeric structures. However, the ionization efficiency of permethylated N-glycans is more than 10 times higher than that of native N-glycans. Also, high temperature separation condition on C18 column allows the separation of several structure isomers. As permethylation largely increases the sensitivity, this approach was further applied to N-glycan profiling of mouse tissue sections. On surface enzymatic digestion was utilized to release N-glycans of mouse tissue sections. Released N-glycans were reduced and on-line purified. This protocol allows the detection of N-glycans derived from RNase B at 10-ng levels. 66 N-glycans were identified from HBS at an injection volume of 0.1 [micro]L. On-tissue PNGase F enzymatic digestion of nude mouse brain tissue sections permitted the detection of 43 N-glycans. In total, the newly developed N-glycan profiling method was found to have high sensitivity and reproducibility and could be widely applied in glycomics studies with minor sample availability.

Keywords: Bioanalytical, Carbohydrates, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Viscosity is an important physical property with widespread relevance in a variety of fields including the material sciences, the petrochemical industry, the food and beverage industry, and the health sciences. We report the development of a microfluidic viscosity sensor that determines viscosity based on the ratio in flow rates of an oil and aqueous phase in a segmented flow stream. This ratio can be determined by continuously measuring the ratio in length of an aqueous segment and oil segment, which provides the capability of doing time-resolved viscosity measurements or single measurements with a large number of averaged ratios. Furthermore, this technique requires no specialized device geometry beyond the simple T-junction already used in many segmented flow devices, which could allow it to be used in conjunction with a number of other existing lab-on-a-chip droplet platforms without any significant changes in design.

Keywords: Lab-on-a-Chip/Microfluidics, Rheology
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Size fractionation and concentration studies of biomolecules <100 nm in size are of utmost interest due to their association with various diseases. There are several methods including electrophoresis, size-exclusion chromatography, membrane filtration, and ultracentrifugation to accomplish size-specific fractionation. However, these methods suffer from sample loss, long analysis times, complex setup, and limited resolution. We designed a nanofluidic-based sieving system devoid of these limitations. Our nanofluidic devices are made using conventional photolithography, sacrificial metal thin films and wet etching. Each device has 200 parallel nanochannels, a height step in each channel, an injection reservoir, and a waste reservoir. When a protein mixture is drawn through the channels by capillary action, proteins smaller than the height step reach the end while larger proteins trap at the height step, resulting in separation. We analyzed the protein size and height step correlation using various concentrations of five different proteins. We also derived a model that can predict size based trapping of proteins in our sieving systems. Finally, we observed alterations in trapping behavior of proteins with changing ionic strength. Our results demonstrate that proteins can be trapped size specifically in these systems. Overall, this study lays the foundation for nanofluidic-based sieving of <100 nm size biomolecules. The potential applications of our devices include lipoprotein fractionation, protein aggregate studies in biopharmaceuticals, and preconcentration.

**Keywords:** Fluorescence, Lab-on-a-Chip/Microfluidics, Protein, Separation Sciences

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Microfluidics: Bioanalytical Chip-western Blotting for Multiplexed Operation

Western blotting is a commonly used assay for proteins. Despite the utility of the method, it is also characterized by long analysis times, manual operation, and lack of established miniaturized counterpart. The use of microfluidics technique substantially increases the separation speed and shows the potential of higher throughput as well as automation. Chip-Westerns with sensitive semi-quantitative detection and about 30 min per assay time were demonstrated before. Briefly, sodium dodecyl sulfate (SDS)-protein complexes are separated by gel electrophoresis in a chip then transferred to a moving membrane so that they are captured in discrete zones. Detection limits for actin, lysozyme, and carbonic anhydrase are 0.7 nM, 4 nM, and 2 nM respectively. This system is also capable of analyzing complex biological samples. AMP-kinase from cell lysates and lysozyme from egg whites are shown to demonstrate versatility of the method. Advances in throughput have been achieved by multiplexing separations for simultaneous immunoassay. We have made 7 parallel separations each connecting to 3 different samples, making the total 21 assays finish in 31 min as illustrated in the figure below.

Keywords: Capillary Electrophoresis, Immunoassay, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
We present a cheap, robust methodology for imaging and sequencing long stranded DNA, stretched out by shear flow in nanoslits to between 80% and 95% of its full contour length. By dying and partially melting a DNA strand the sequence of the gene is revealed. Subsequent stretching the gene allows for easy imaging of the structure of the molecule. We have performed experiments in cheap, disposable, injection molded polymer chips, created from a single master wafer, produced in cleanroom facilities. This production scheme greatly reduces the costs of the individual chip. Because of the low cost of the chip, we can change the chip between each experiment, reducing the chance of cross contamination between samples. The material used for fabricating the chip is optimized to reduce the level of background signal. By proper use of additives in the polymer, we have reduce the background signal to a level comparable to glass chips, allowing a high signal to noise ratio. Classical DNA sequencing relies heavily on fractionation and amplification of the target, making it difficult to identify repetitions, deletions, and insertions in the genome. Furthermore the increase in workload increases the cost of the experiment. By clever chip design and the use of an induced flow, we stretch out the DNA molecule causing it to un-scroll, allowing optical linear analysis. The present chip design allows for mapping of up to 1.3Mbp making it easy to see if the gene includes any anomalies, such as deletions, insertions or repetitions.

The research has been funded by the Danish Council for Strategic Research through the Strategic Research Center PolyNano (grant no. 10-092322/DSF) and the Danish Agency for Science, Technology and Innovation through DELTA’s performance contract (grant number 10-076609)
## Abstract Text

The dysregulation of cell-to-cell signaling is implicated in a wide range of human health conditions, from metabolic to neurological disorders, and others. Next-generation biosensors will elucidate mechanisms of disease by measuring hormones and neurotransmitters via highly selective interactions with native membrane receptors. Here, recent progress is reported towards a new biosensor platform technology based on ion channel-coupled receptors (ICCRs): a class of engineered transmembrane proteins that couple G protein coupled receptors (GPCRs) to ion channels for highly selective and sensitive measurements of ligand-GPCR interactions. A new SU-8 fabrication procedure will be described which yields microapertures with diameters as low as $7 \mu m$, and reproducible, well-defined, three-dimensional geometries. These apertures facilitate the self-assembly of black lipid membranes (BLMs) with sufficient electrical stability (breakdown voltage $400 - 600$ mV) and longevity (lifetimes $> 2$ hrs) to support continued sensor development. Low dead volume coupling of BLMs to microfluidic flow channels was accomplished with a novel hybrid SU-8/PDMS microfluidic device. The hybrid device facilitated studies of membrane physiology by simultaneous electrophysiology and epifluorescence microscopy. Electrophysiology studies of ICCR-expressing cells have demonstrated chemical-to-electrical signal transduction by ICCR-ligand binding. Approaches to integrating ICCRs with the BLM microdevices will be described. The resulting biosensor technology will be readily adapted to measure a myriad of GPCR-ligand interactions, facilitating studies of a wide range of disease mechanism and enabling identification of GPCR-targeting drug candidates.

### Keywords:
- Bioanalytical
- Biosensors
- Lab-on-a-Chip/Microfluidics
- Nanotechnology

### Application Code:
- Bioanalytical

### Methodology Code:
- Integrated Sensor Systems
Microfluidics: Bioanalytical

On-Line Microdialysis-Microchip Electrophoresis with Electrochemical Detection for the Study of the L-DOPA Metabolic Pathway

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**Abstract Text**

Microdialysis is a widely applied [i]in vivo[/i] sampling technique used to monitor extracellular concentration changes in the brain. Off-line analysis with conventional methods is most commonly employed for the analysis of microdialysis samples. However, this can lead to a loss of valuable temporal information concerning dynamic processes due to the large sample volumes necessary for more traditional analysis methods. In order to preserve temporal information regarding concentrations, the ideal analysis system is one that can be employed on-line, is fast, and has the ability to analyze very small sample volumes. Microchip electrophoresis is suited to this type of analysis, and incorporates the possibility of integrating electrochemical detection directly on-chip. In this study, a simple approach for coupling microdialysis to microchip electrophoresis with electrochemical detection at a carbon electrode is described. The device consists of a double T PDMS/glass hybrid microchip utilizing electrokinetic gating with a 5 cm separation channel and in-channel detection at two pyrolyzed photoresist carbon electrodes in series. The separation of L-Tyr, L-DOPA, N-Tyr, HVA, DOPAC, and dopamine was achieved in less than 100 s [i]in vitro[/i]. This device was then used on-line to monitor the transport of L-DOPA across the blood-brain barrier, its conversion to dopamine, and subsequent metabolism [i]in vivo[/i] in an anesthetized rat.

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

**Application Code:**  
Bioanalytical

**Methodology Code:**  
Microfluidics/Lab-on-a-Chip
Nitric oxide (NO) is involved in many physiological processes including neurotransmission, vasodilation, and inflammation. In particular, macrophages are known to produce nitric oxide as part of the immune response. Different phenotypes of macrophages are known to exist, including M1 and M2, which are pro-inflammatory and anti-inflammatory, respectively. We have previously detected intracellular NO in macrophage bulk cell lysates following stimulation with lipopolysaccharide using microchip electrophoresis with electrochemical detection (ME-EC). However, in order to differentiate the NO producing capabilities of macrophage phenotypes in an identical culture, a single cell system is necessary. In this presentation, progress toward the development of a single cell analysis system using ME-EC is described. For single cell analysis, a faster separation time and lower detection limits will be necessary, to achieve higher throughput and compensate for signal suppression due to matrix effects. Using a 3.5 cm separation channel and field strength of 400 V/cm, the separation of nitrite, NO, and cellular interferences was achieved in 25 seconds. Transient isotachophoresis was also investigated to obtain lower limits of detection, and a system consisting of chloride, phosphate, and sodium as the leading electrolyte, tailing electrolyte, and counterion, respectively, was developed. A greater than 10-fold decrease in the limit of detection was achieved under these conditions. The application of these modifications will result in a ME-EC system that is ideally suited for the investigation of the effect of macrophage phenotype on NO production.

Thank you NINDS and the Madison and Lila Self Graduate Fellowship for support.

Keywords: Bioanalytical, Electrochemistry, Electrophoresis, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Microfluidics: Bioanalytical Frequency Encoded Fluorescence for the Reduction of Optical Complexity in Microfluidic Devices

Polymerase chain reaction has been one of the most influential tools in the field of genetic analysis. One of the many improvements since its inception has been the use of non-contact heating methods, such as infrared mediated PCR. Only recently has quantitative IR-PCR (IR-qPCR) been realized. One of the drawbacks of real-time IR-qPCR is the excess light present in the system due to the IR lamp. We present a novel method to discriminate fluorescent signals from the background IR lamp radiation. Frequency modulated fluorescence encoding was used to increase the number of fluorophores monitored during infrared-mediated polymerase chain reaction on a single detector. The fluorescence of a DNA intercalating dye and a passive reference dye were encoded by pulsing the exciting lasers at 73 and 137 Hz. The resulting signals were demodulated by Fourier methods resulting in the individual fluorescent traces isolated from background IR. The method was used to successfully detect amplification of a 309 bp region from PUC19 and a calibration curve indicated an amplification efficiency of 96%. The method was further used to demonstrate real-time monitoring of a multiplexed Taqman IR-qPCR of two house-keeping genes from genomic DNA. This method of multiplexing fluorescence with IR-qPCR is ideally suited as it allows the isolation of signals of interest from the background IR as well as requiring less detectors and optics than other multicolor detection methods. This method is general enough to be used as a means to reduce the optical complexity of other multiplexed microfluidic assays.

Keywords: Detection, Fluorescence, Lab-on-a-Chip/Microfluidics, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Adenosine is a purine nucleoside, composed of adenine attached to a ribose. The major therapeutic uses of adenosine are for treating surgical and nerve pain, pulmonary hypertension, irregular heartbeat, controlling blood pressure during anesthesia/surgery, and for cardiac stress tests. For these indications, adenosine is administered either as a bolus intravenous injection or as an intravenous stress infusion.

Commercially, adenosine is produced by the condensation of ribose and adenine chemically, biochemically, or by fermentation. Ionic impurities that could be present in adenosine preparations are ammonia, chloride, and sulfate. The adenosine monograph in the United States Pharmacopeia (USP35-NF30) currently has color comparison assays for these ions. The color assays are outdated, imprecise, and are subjective assays that potentially expose the analyst to mercury and create hazardous waste. There is an ongoing effort by the USP to review monographs for modernization, which includes updating analytical tests, and eliminating or minimizing the use of hazardous chemicals.

In the spirit of monograph modernization, we developed ion chromatography (IC) assays for ammonia, and for chloride and sulfate that meet the requirements of the adenosine monograph. Ammonia, as ammonium, was separated on a high-performance cation-exchange column, while the anions were separated on a hydroxide-selective anion-exchange column; columns designed for fast separation of ions. Both the assays require < 5 min per sample, are sensitive, and accurately determine the ions by suppressed conductivity detection. The precisions (retention time RSD < 0.1%, peak area RSD <2.3%), accuracy (average recovery 88-110), and limits of detection, meet the analytical performance characteristics suggested by USP General Chapter <1225>.

Keywords: Chromatography, Ion Chromatography, Liquid Chromatography, Pharmaceutical
**Determination of Morpholine in Linezolid by Ion Chromatography**

Morpholine is a commonly used compound in organic synthesis. Substituted morpholine derivatives are the core of various natural products and biologically active compounds. Morpholine is used as a building block in a multistep synthesis to produce linezolid, which is a synthetic antibiotic of the oxazolidinone class. Because morpholine is used in the preparation of the linezolid, it can remain as an impurity in the final product. The level of impurities in a drug substance or product must be carefully controlled and monitored because impurities can diminish the pharmacological efficacy of the active pharmaceutical ingredient or cause unwanted side effects. Therefore, a sensitive method is needed to determine morpholine in synthetic drugs such as linezolid.

Although linezolid and its degradation products are usually assayed by HPLC with UV detection, morpholine lacks a suitable chromophore for detection. This study demonstrates an ion chromatography method for determining low concentrations of morpholine in linezolid. Sample solutions of 0.1 mg/mL linezolid were constituted in 10% methanol to speed the dissolution of linezolid. To prevent column contamination by linezolid, in-line matrix elimination was performed to concentrate trace morpholine on a concentrator column, followed by removal of the uncharged linezolid and methanol with DI water using an autosampler. The separation was achieved on a cation-exchange column using electrolytically generated high-purity methanesulfonic acid eluent. Trace morpholine was detected using suppressed conductivity. The method was validated with intraday and between-day recovery studies on spiked linezolid samples, which were in the range of 98.8-101% and 99.9-103%, respectively.

**Keywords:** Ion Chromatography, Pharmaceutical, Trace Analysis

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography
Salt formation is important in drug development to improve biopharmaceutical and physicochemical properties of the drug. Approximately 50% of all drugs are formulated as salt forms. A broad selection of inorganic and organic ions can be used as pharmaceutical counterions. Because detection sensitivity is not challenging when analyzing pharmaceutical counterions, ion analysis can be performed on an ion-exchange LC column (e.g., amino and sulfonate phases) using an HPLC system provided that a suitable detector is available. However, for such a system, anions and cations need to be analyzed separately using different methods, different separation columns, and very often, different instruments. It is highly desirable to be able to determine both pharmaceutically important anions and cations on the same separation column, using an HPLC system, and within a single analysis. This presentation will describe a platform method for screening all pharmaceutical counter ions (e.g., sodium, potassium, magnesium, calcium, chloride, bromide, nitrate, malate, citrate, sulfate, fumurate, citrate, etc). This separation solution, based on advanced column and detection technology, includes one separation column, one common mobile phase system, one LC system, and one simple chromatographic condition. Examples of pharmaceutical counter ion screening and simultaneous detection of drug substance and counter ion will be discussed.

Keywords: HPLC Columns, HPLC Detection, Liquid Chromatography, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Amlodipine besylate is a calcium channel blocker that is used for the treatment of hypertension and angina. Similar to other calcium channel blockers, amlodipine works by relaxing the arterial muscles, which decreases peripheral resistance and therefore reduces blood pressure. Active pharmaceutical ingredients (APIs), such as amlodipine, are commonly manufactured into acid addition salts to improve bioavailability. Amlodipine besylate is the salt form of amlodipine, which is produced from the reaction of amlodipine (a weak base) and benzene sulfonic acid. It is critical to quantify the concentration of the API and counter-ion in the drug formulation to establish stoichiometry, completeness of salt formation, and mass balance. We developed an isocratic ion chromatography (IC) method with suppressed conductivity detection for determining besylate in amlodipine besylate. A second method was developed for the simultaneous determination of amlodipine and besylate using a tri-mode (reverse-phase, anion exchange, cation exchange) HPLC column with UV detection. The HPLC method used a mobile phase containing 70% 100 mM ammonium acetate/30% acetonitrile. The recovery of besylate spiked into the API ranged from 99-104% using the IC method. In addition, the amount of besylate in amlodipine besylate was about 28.5% with the IC method and 29.0% with the HPLC method, which is comparable to the theoretical amount of 27.9% based on the molecular weight of the drug formulation. These results demonstrate the ability to accurately determine the counter-ion in a drug formulation by IC with suppressed conductivity detection. In addition, comparable results can be obtained for the counter-ion when using HPLC with UV detection. The HPLC method also provides additional advantages by simultaneously determining the API and counter-ion, which is a cost effective approach for many laboratories. The two methods are shown to have good linearity, sensitivity, precision, and accuracy.

Keywords: HPLC, Ion Chromatography, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Ion analysis is important for pharmaceutical industry because many active pharmaceutical ingredients (APIs) exist in their salt form. Pharmaceutical products are strictly regulated by the United States Food and Drug Administration (U.S. FDA) and other regulatory agencies, and must be tested for composition to verify their identity, strength, quality and purity. Recently, identification and quantification of ions in early stage drug development has gained increasing attention, because the APIs maybe contaminated with different counter ions from synthesis steps, and because selecting the counter ion to enhance APIs’ solubility and stability is becoming a key step in formulation development. Since many of the ions are non-chromophoric, Ion chromatography (IC) with suppressed conductivity detector (CD) is an established method used for ion determination. The recently introduced high-pressure capable capillary IC systems (HPIC) combined with 4-µm particle ion-exchange columns have improved separation efficiency and significantly reduced the eluent consumption to 5.2 L/year which saves money and time. Use of a newly invented charge detector (QD) combined with CD permits peak purity assessment to further simplify identification and confirmation of ions.

This study demonstrates the identification and quantification of 22 commonly found anions in pharmaceuticals in a single run using a high-pressure capillary IC system (HPIC) with 4 µm particle ion –exchange column, and CD/ QD dual detectors.
A Rapid Novel Gel Filtration Solution for Determining Protein Aggregation

Quantitating the amount of aggregated protein in a protein therapeutic drug is one of the required tests for analyzing a therapeutic protein product and one of the most deleterious of post translational modifications that can occur. Aggregated proteins can illicit potentially lethal immune responses including neutralizing antibodies and anaphylactic shock and thus a major concern for any biopharmaceutical. A new methodology and chromatography solution is presented in this presentation that provides a more accurate method for quantitating aggregate in protein solution. This method is also faster than most other column assays and has reduced issues with non-specific binding and column priming that other columns demonstrate. Strategies for rapid method development using such column technologies will be presented.

Keywords: Biopharmaceutical, HPLC, Protein
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Separation of Nucleotides by Hydrophilic Interaction Chromatography (HILIC) Using the FRULIC-N Column

A stationary phase composed of silica-bonded cyclofructan 6 (FRULIC-N) was evaluated for the separation of four cyclic nucleotides, six nucleoside monophosphates, four nucleoside diphosphates, and five nucleoside triphosphates via hydrophilic interaction chromatography (HILIC) in both isocratic and gradient conditions. The gradient conditions gave significantly better separations, by narrowing peak widths. Sixteen out of nineteen nucleotides were baseline separated on the FRULIC-N column in one run. Unlike other known HILIC stationary phases, there can be dual retention mechanisms unique to this media. Traditional hydrogen bonding / dipolar interactions can be supplemented by dynamic ion interaction effects for anionic analytes. This occurs because the FRULIC-N stationary phase is able to bind certain buffer cations. The extent of the ion interaction is tunable, in comparison to stationary phases with embedded charged groups, where the inherent ionic properties are fixed. Optimization approaches were examined by varying the organic modifier (acetonitrile) content, as well as salt type/concentration and electrolyte pH. The thermodynamic characteristic of the FRULIC-N column was investigated by evaluating the column temperature effect on retention and utilizing van’t Hoff plots.

Keywords: Chromatography, HPLC, HPLC Columns, Liquid Chromatography
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
The recent progress in core-shell particle technology brings to chromatographers unparalleled resolving power for their increasingly demanding and challenging separation needs. These particles enable the development of analytical methods with shorter run times and higher sensitivity. In cases where analytes of interest are very closely eluted, the sheer power of efficiency might not be enough to provide adequate resolution. Some examples are very polar analytes typically difficult to retain in RPLC or geometric isomers and stereoisomers which can be very similar in chromatographic behavior. In such cases only appropriate selectivity of the stationary phase can ensure adequate resolution. Therefore, an effective toolbox of stationary phases with high resolving power and orthogonal selectivity is critical to satisfy the needs of today’s method developer. The purpose of this study is to demonstrate the advantages of coupling the core-shell technology with surface modification that can provide adequate selectivity even in difficult separations such as the ones mentioned above.

Keywords: HPLC, HPLC Columns, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Analytical tools with high temporal sensitivity and chemical specificity are required to further the understanding of chemical dynamics at biologically relevant interfaces. The signal enhancement associated with surface enhanced Raman spectroscopy (SERS) makes SERS a strong candidate for the interrogation of biomembranes in a label-free fashion. Toward this end, we demonstrate detection and correlation analysis for macromolecular assemblies interacting with a highly enhancing SERS substrate. Spectral acquisition on the millisecond timescale allows for monitoring of particle diffusion dynamics through auto- and cross-correlation analysis. Surface enhanced Raman correlation spectroscopy (SERCS) shows promise for the analysis of multiple targets simultaneously and label-free study of chemical organization and dynamics in biomembrane systems.
The excitation of plasmon resonances creates local electric fields that increase sensitivity in chemical sensing. The vibrational Stark effect has been used to investigate local electric fields that are present in biological and interfacial systems. To that extent, little has been done in the way of determining the electric fields associated with localized surface plasmon resonances by Stark chromophores. The enhancement factor in surface enhanced (SERS) and tip enhanced (TERS) Raman spectroscopy is inferred from the changes in Raman intensity. The vibrational Stark Effect provides a direct measurement of the electric field experienced by molecules. It was previously reported that the stark shift associated with tip enhanced Raman was very weak. We have found that improved overlap between the CN stretch Raman frequency and the excited plasmon resonance can produce a large Stark shift from probe molecules in the enhanced electric field. We have been able to show this through SERS from micro-electrodes which show a blue shift in the cyanide peak that correlates with an increased SERS intensity of co-deposited thiophenol. TERS results show even more dramatic shifts, suggesting enhancement factors on the order of $10^{13}$ in these experiments. To expand on these findings, Stark tuning rates of cyanothiols in similar fields generated from closely spaced electrodes have been measured. These measurements were performed with the use of AFM and nano-electrodes with no SERS properties present.

Keywords: Electrode Surfaces, Imaging, Raman, Surface Enhanced Raman
Application Code: Nanotechnology
Methodology Code: Molecular Spectroscopy
Functionalized nanoparticles are widely used for many applications including detection of metal ions by different techniques including colorimetric and surface enhanced Raman scattering, SERS. In general, for the functionalization, the appropriate sensing molecule needs to be identified for the target. In order to improve the sensitivity of detection by using SERS, the nanoparticles must have a second molecule known as a reporter attached to nanoparticles. This shows that an interaction occurred by ‘switching on’ the SERS signal due to the formation of hot spots following aggregation of the nanoparticles. This occurs since the SPR band of the nanoparticles is sensitive to the surrounding environment, hence any changes occurring at a molecular level cause changes to the SPR band resulting enhancement of Raman scattering from the reporter molecule.

In this study 4-mercaptobenzoic acid (4-MBA) was used as both the sensor ligand and reporter to specifically detect Cu2+ ions at very low concentration. 4-MBA is a commonly used SERS reporter which gives a very intense SERS signal and more importantly, Cu2+ ions form a very stable complex with the carboxylic acid group of the MBA molecule. Cu2+ ions play an important role in human biology however, at elevated concentrations they are highly toxic species that cause severe damage to the human body. Therefore, the detection and quantification of Cu2+ ions has become increasingly important. Many methods exist for the detection of Cu2+ ions and here we report a new SERS method that is simple and more sensitive than the more commonly utilised colorimetric methods. This work also leads to the potential for multiple ion detection by SERS using differently functionalized nanoparticles.

**Keywords:** Molecular Spectroscopy, Quantitative, Raman, Surface Enhanced Raman

**Application Code:** Quality/QA/QC

**Methodology Code:** Molecular Spectroscopy
We have synthesized a bimetallic (Ag-Au) nanodendrite structure and used it as a SERS platform for the detection of DNA point mutation, based on DNA-displacement reaction. Dendrites have a hierarchical structure with high population of edges as well as acute angles in terraces; therefore, there are many hot spots within the structure, which could enhance the sensitivity of SERS measurement. Initially, the hybridized pair of probe DNA (complementary to a point mutated DNA) and indicator DNA labeled with Raman dye (shorter than probe DNA) was immobilized on the surface of Ag-Au nanodendrite, thereby resulting in the increase of Raman signal due to the positioning of the Raman dye near the surface. When a target DNA was added, it pushed the indicator DNA away, so decreasing Raman intensity as a function of the concentration. In overall, the current research demonstrate the first SERS-based measurement utilizing DNA displacement reaction.

Keywords: Bioanalytical, Sensors, Surface Enhanced Raman
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Surface-enhanced Raman scattering (SERS) has been attracted enormous attention from the scientific community due to its high sensitivity and very specific molecular information it can provide in a very short time. However, it suffers from irreproducibility due to the fact that molecules or molecular structures should be near to or in contact with the surface of a nanostructured noble metal. The use of SERS has generally been investigated in two contexts; in an assay as replacement of existing technique such as fluorescence or molecular characterization. The studies implementing of the latter approach is also on the rise. In this study, we have evaluated the performance of SERS for possible quantitative and qualitative applications in biomedicine from protein detection and identification to cancer diagnosis without using an external label. We have found that although the technique has great potential for development of new approaches for either replacement of an existing conventional technique or in support of a current technique, it has also fundamental limitations. In this presentation, the strength and the limitations of the technique are presented with the data obtained from proteins to living cells to tissue in our laboratory.

The author acknowledge the financial support from The Scientific and Technological Council of Turkey (TUBITAK) (Project No: 105T135 and 109T941) and Yeditepe University.
A Non-Destructive Optical Method for the Simultaneous Determination of Physical and Chemical Properties of Biomaterials

The lack of non-destructive techniques that yield physically and chemically relevant information in a single pass has limited progress in many fields of materials science and biomaterials research. Optical inelastic scattering spectroscopy provides a non-destructive approach for simultaneously measuring key physical and chemical properties of a sample. Inelastic scattering results from the interaction of incident photons with sample derived phonons. Low frequency acoustic phonons can provide physical details of a sample such as Young’s Modulus and are interrogated using Brillouin detection. High frequency optical phonons cause a greater wavelength shift in the scattered light and are measured using a Raman spectrometer. Hence integration of Brillouin and Raman detection modalities enables both physical and chemical information to be obtained from the sample.

In the work presented here, we describe a new method for simultaneously obtaining Brillouin and Raman spectra. The Brillouin scattered light generally resides within 5 wavenumbers of the incident light and is resolvable using single frequency laser illumination and a virtually imaged phased array (VIPA) spectrograph. A laser rejection filter placed in the scattered light path diverts the Rayleigh and Brillouin scattered light to the VIPA-based spectrograph while transmitting the Raman component into the entrance slit of a dispersive Raman spectrometer. An inherent advantage of the dual collection path geometry is that no sample registration errors exist between the Brillouin and Raman data sets. In addition to the instrument design, we present preliminary data obtained using the new method and describe the advantages and limitations of the technique.

Keywords: Imaging, Instrumentation, Materials Characterization, Raman
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Poly-L-lactide (PLLA) is a biodegradable and biocompatible implant polymer that has been utilized as an implant material and drug release substrate. The elastic properties of PLLA vary depending on the degree of its crystalline character. Cold-drawing PLLA at temperatures above the glass-transition temperature and below the cold-crystallization temperature induces molecular ordering. By controlling the draw ratio, PLLA substrates can be designed to conform to a specific elastic moduli and crystalline character, on average, making them better suited for a particular application or target tissue type.

In the work presented here, we describe a high fidelity wide-field Raman imaging system and an accompanying chemometric method for elucidating the localized crystalline content across the cold-drawn PLLA surface. Our implementation overcomes some of the previous limitations of acousto-optic tunable filter (AOTF) imaging systems and combines AOTF-based acquisition with novel multivariate strategies based on Gram-Schmidt orthogonalization and spectral identity mapping (SIM). Because the AOTF diffracted light is linearly polarized, it is important to understand the way in which the Raman spectrum is affected by laser polarization, sample orientation, and draw ratio. To answer these questions, we have carried out Raman polarization studies on PLLA substrates with different draw ratios. The Raman polarization and wide-field image results are presented, and reveal that the orientations of bonds comprising the polymer chain backbone are preferentially affected by cold drawing as expected. In addition to these results, the instrument design and a theoretical description of the multivariate methods employed are presented.

**Keywords:** Chemometrics, Imaging, Raman, Spectroscopy

**Application Code:** Other (Specify)

**Methodology Code:** Vibrational Spectroscopy
### Sample Preparation: Environmental Water Analysis

#### New Method US EPA 625 with Solid Phase Extraction for Challenging Wastewaters

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.

In this paper we will describe the use of solid phase extraction, rather than liquid/liquid extraction, for preparing wastewater samples for GC/MS analysis. The extract will be dried using an alternative to sodium sulfate, a membrane for physical separation of water. The quality control criteria currently in method 625 will be used for evaluation of the new method options with real and challenging samples.

**Keywords:** Sample Preparation, Solid Phase Extraction, Water

**Application Code:** Environmental

**Methodology Code:** Sampling and Sample Preparation
Sample Preparation: Environmental Water Analysis

Ultraviolet Photoinitiated On-Fiber Copolymerization of Ionic Liquid Sorbent Coatings for Headspace and Direct Immersion Solid-Phase Microextraction

Within the past decade, solid-phase microextraction (SPME) has enjoyed increasing popularity in the fields of sample preparation. To increase the sensitivity and selectivity for target analytes, polymeric ionic liquids (PILs) have been applied as sorbent coatings in SPME. However, the fabrication of PIL-based coatings can be tedious and time consuming. We report the high-throughput fabrication of ultraviolet (UV) photoinitiated crosslinked polymeric ionic liquid (PIL) sorbent coatings for SPME. The crosslinked PIL-based sorbent materials were produced “on-fiber” by the copolymerization of monocationic and dicationic IL cross-linkers in the presence of a UV initiator. Prior to copolymerization, the fused silica is etched and derivatized to facilitate the covalent bonding of the sorbent coating to the fiber surface. Production of the UV-initiated PIL-based coatings requires no organic solvents and is more rapid compared to traditional thermal-initiated routes using 2,2'-azobis(2-methylpropionitrile) (AIBN). Various polar cross-linked PIL-based coatings were fabricated from the copolymerization of one IL cross-linker, either 1,8-di (3-vinylimidazolium) octane dibromide \([(\text{VIM})_2\text{C}_8\text{Br}_2]\) or 1,12-di (3-vinylimidazolium) dodecane dibromide \([(\text{VIM})_2\text{C}_{12}\text{Br}_2]\) with an IL monomer, namely, 1-vinyl-3-hexylimidazolium chloride \([(\text{VHIM})\text{Cl}]\). The crosslinked PIL-based coatings were applied for the extraction of various polar compounds from simple and complex water matrixes in both direct immersion and headspace sampling modes. The fabrication, method development, analytical performance, and method validation for the novel UV-initiated crosslinked PIL-based coatings will be presented herein.

This work is funded by the National Science Foundation, Division of Chemistry (Analytical and Surface Chemistry Program, CHE-0748612).

Keywords: Environmental/Water, Sample Preparation, SPME, Water
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Sample Preparation: Environmental Water Analysis

On-Line Preconcentration of Haloacetic Acids for Analysis by Post-Column Reaction-Ion Chromatography with Nicotinamide Fluorescence in Drinking Water

Haloacetic acids (HAAs) are a class of chlorinated disinfection by-products produced during the chlorination of drinking water. The HAAs have potentially adverse health effects and are regulated by the United States Environmental Protection Agency (USEPA). The maximum contaminant level for HAAs is 0.060 mg/L. The original post-column reaction-ion chromatography with nicotinamide fluorescence (PCR-IC) analyzer had method detection limit (MDL) values that ranged between 1 – 10 [micro]g/L.

A sequential injection analysis (SIA) module has been developed for fully automated preconcentration of HAAs prior to analysis by PCR-IC. SIA is a technique that uses discontinuous flow to minimize reagent usage and also pass multiple reagents through a single flow path. Here, the preconcentration of HAAs is achieved with solid phase extraction (SPE) with LiChrolut EN cartridges, a resin designed for environmental analysis. The HAAs are first protonated with sulfuric acid, preconcentrated on the SPE cartridge, and eluted with a sodium hydroxide solution. Optimization of method parameters will be presented along with detailed MDL, accuracy, precision, and linearity studies.

Side-by-side comparison studies of HAAs analysis in real-world drinking water samples are also presented that compare the optimized SIA-PCR-IC method and USEPA 552.3. Trace levels of HAAs detected in the samples are reported and the bias values calculated between the two methods are acceptable overall. Additional studies were performed to incorporate on-line internal standardization and sampling, which successfully decreased system maintenance and method error.

Keywords: Environmental/Water, Ion Chromatography, Method Development, Sample Handling/Automation

Application Code: Environmental

Methodology Code: Sampling and Sample Preparation
The chlorination of water is the most commonly used process for water disinfection in the United States, but leads to the formation of disinfection by-products (DBPs). DBPs have been known to cause adverse health effects. Two major classes of DBPs that are regulated by the United States Environmental Protection Agency (USEPA) are trihalomethanes (THMs) and haloacetic acids (HAAs).

The USEPA has several standard methods for compliance monitoring of THMs and HAAs. These methods have excellent method detection limits and are high in accuracy and precision. However, these standard methods are expensive and require skilled analysts. For these reasons many water treatment plants do not have access to these methods.

Recently, a portable disinfection by-product detection kit has been created to provide process monitoring of Total THMs and Total HAAs between 20 – 120 ppb using standard addition. However at the distribution plant, operators need to measure concentrations of THMs and HAAs that are near 5 ppb or less. Semi-automated preconcentration methods were developed to enhance the method detection limit, accuracy and precision of the original disinfection by-product detection kit. The preconcentration method uses standard addition and solid phase extraction techniques with the capillary membrane sampling device to separates THMs from HAAs in drinking water. The preconcentrated sample and spikes are mixed with reagents, heated, cooled then read on a handheld fluorimeter. Optimization of the preconcentration steps, MDL, accuracy, and precision for the improved kit will be presented along with side-by-side comparisons in real-world drinking water samples.

Keywords: Environmental/Water, Fluorescence, Portable Instruments, Water
Application Code: Environmental
Methodology Code: Portable Instruments
Interest in the analysis of steroids and other drugs in water has increased because they have been detected in ultra-trace (part per trillion and lower) quantities and are suspected of having adverse effects on aquatic life. Analytical techniques that have been employed for this analysis include LC-MS, UV spectrometry and traditional GC-MS. With traditional one-dimensional GC-MS, problem is the co-elution of steroids with each other and with matrix components is a challenge. In this work steroids were extracted from water using direct immersion solid phase microextraction (SPME) using polydimethyl siloxane-divinyl benzene (PDMS-DVB) coated fiber and analyzed using GC-MS-MS. The developed analytical method was validated and method was linear from 0.01 to 5 ng/ml with linear regression coefficient greater than 0.99 and precision less than 10% for all the steroids at 1 ppb. Limit of quantitation and limit of detection was found to be in ppq levels and the analysis was extended to real water samples. To determine partition coefficient, a depletion study was performed for 8 different steroids with direct immersion SPME. This involved generating a plot of logarithm of peak area against the extraction number. The linear regression coefficient for the depletion study plot was found to be more than 0.99. The fiber/liquid partition coefficient was found to be in thousands which demonstrated high extraction efficiency of the method and fiber. Additionally, no internal standard or derivatization was used for this work.

Keywords: Environmental/Water, Gas Chromatography/Mass Spectrometry, SPME, Tandem Mass Spec
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
A high-throughput method based on thin-film solid phase microextraction (SPME) and liquid chromatography mass spectrometry was developed for simultaneous quantitative analysis of 9 benzylic and aliphatic quaternary ammonium compounds. Chromatographic separation was obtained in reversed-phase mode in 8 min method; using triple quadrupole mass spectrometer. Hydrophilic lipophilic balance (HLB) particle coated blades were found to be the best of different coatings tested in terms of recoveries and carryover on the blades. The SPME method was optimized in terms of extraction pH, preconditioning, extraction and desorption times. Wide linear dynamic ranges with the developed method were obtained for each compound, which enables application for a wide range of concentrations. The developed method was validated according to the Food and Drug Administration (FDA) criteria. Dual nature of the quaternary ammonium compounds; having permanently charged hydrophilic quaternary ammonium head and long chain hydrophobic tail, makes the sample preparation step and analysis of these compounds challenging. In this study it was found that for analysis of these compounds special care is required for treatment of labwares, desorption conditions as well as chromatographic conditions. In this presentation detailed discussion and special attention will be given to the method development to overcome problems regarding adsorption losses during the sample preparation step as well as carryover on coating and LC-MS system.

Keywords: Environmental/Water, High Throughput Chemical Analysis, SPME, Tandem Mass Spec
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
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. 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 to have a simple and automatic technique which allows the fast analysis with an 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, its necessary to test as alternative gas, Argon which is readily available. This paper presents data on Nitrogen determination in soils and plants reference materials with different Nitrogen concentration to show the performance of the system using Argon as carrier gas and the reproducibility of the results obtained.

Keywords: Agricultural, Elemental Analysis, Method Development, Soil
Application Code: Agriculture
Methodology Code: Other (Specify)
Trace Analysis of Glycine and its Methylated Derivatives in Small Volume of Plant Fluids by Surface-Enhanced Raman Scattering with a Cylindrical SERS Substrate

The levels of glycine and its methylated derivatives in biological systems play crucial roles in the cellular metabolic pathways and the responses to environmental stresses. To determine the levels of these glycine-derived metabolites, a rapid and sensitive method is urgently needed as a method based on surface-enhanced Raman spectroscopy (SERS) was proposed herein. With a sensitive cylindrical SERS substrate, plant samples can be analyzed non-destructively and the required volume of sample is less than few microliters. To prepare the SERS substrates, silver nanoparticles (AgNPs) were placed onto the surface of the tip of polymethyl methacrylate (PMMA) optical fiber array by silver mirror reaction. Through proper treatment of the surface tips, aqueous sample can form a water drop on the tip of each fiber and the required volume of sample could be as low as 1 μL with no need for a drying procedure. Meanwhile, the formed SERS fiber array allows mass analysis as each optical fiber in the fiber array works as a single SERS substrate. Use the optimized preparation condition, this array SERS substrate offers an enhancement factor around 7 orders in magnitude with a relative standard error less than 10%. Using the optimized condition in preparation of the fiber array, glycine and its derivatives could be detected with limits approaching to ppb in only a few microliters of solution.

Keywords: Amino Acids, Biological Samples, Nanotechnology, Raman
Application Code: Agriculture
Methodology Code: Vibrational Spectroscopy
The characterization of flavor analytes in complex natural products, such as tobacco, can be important for quality control and process optimization. Methods were developed for sample preparation, chromatographic separation, and mass spectral detection to analyze natural flavor analytes in cigarette tobacco. Tobacco was removed from cigarettes and the volatile and semi-volatile compounds were sampled with headspace solid phase micro-extraction (HS-SPME) using a divinylbenzene/carboxen/polydimethylsiloxane fiber (50/30 um DVB/CAR/PDMS, Supelco, Bellefonte, PA, USA). A two-dimensional gas chromatography (GCxGC) coupled to time-of-flight mass spectrometry (TOFMS) method was developed to isolate and identify individual analytes within the complex tobacco matrix, including flavor compounds known to occur naturally in tobacco. These techniques provide a reliable method to locate, identify, and quantify tobacco flavors.
Agriculture

Determination of Rare Earth Elements in Tea Leaves by ICP-AES with Ultrasonic Aerosol Generator

With the food quality and security being paid more attention, food quality is also being managed more and more strictly. Chinese National Standard for Contaminants Limitation in Food (GB 2762-2012) was released in 2012. The standard set the maximum allowable amounts of total amount of oxide of rare earth elements (REEs) in tea leaves (<2.0 mg/kg). A method for determination of REEs (Ce, Er, Eu, Gd, Ho, La, Lu, Nd, Pr, Sm, Tb, Tm, Y, Yb, etc.) using ICP-AES with ultrasonic aerosol generator was established. Ultrasonic aerosol generator was used to increase sensitivity of ICP-AES for more than 3 times, compared to a conventional nebulizer. Tea leaves samples were digested with HNO3-HClO4 mixed system by wet digestion. Quantitative analysis was conducted with standard addition method. Under the optimum conditions, the limits of detection of REEs in tea leaves were lower than 0.005 mg/L. The recoveries of REEs at 0.02 mg/L ranged from 90% to 106.0%. Accuracy of this method was evaluated by measuring the China tea standard reference material GBW10016, and the results matched the certified values well. With high accuracy and good precision, this method meets the requirement for Chinese regulation of trace determination of REEs in tea leaves.

Keywords: Agricultural, Elemental Analysis, ICP
Application Code: Agriculture
Methodology Code: Atomic Spectroscopy/Elemental Analysis
A rapid quantification method was developed and validated for simultaneous and nondestructive measurement of starch content and ethanol yield of 44 cultivars of sorghum grain using Fourier transform near infrared (FT-NIR) spectroscopy in diffuse reflectance mode. Multiplicative scatter correction (MSC), the first derivative of Savitzky-Golay, and mean centering were used as spectral processing options. Starch content in sorghum flour (milled through a 1 mm screen) was calculated from determined glucose content after acid hydrolysis. Ethanol was produced through simultaneous saccharification and fermentation process and the final yield was determined by High-performance liquid chromatography (HPLC). Calibration models were established by the partial least squares regression (PLSR) analysis. Spectral range and the number of PLS factors were optimized for the lowest root mean square error of prediction (RMSEP), coefficient of determination ($R^2$), and ratio of performance to deviation (RPD). The best models showed satisfactory predictions as measured by the RMSEP, $R^2$, and RPD values: starch content, 1.21 % (w/w), 0.87, 2.75; ethanol yield, 0.59 g/L, 0.73, and 1.92, respectively. FT-NIR analysis is a practical method for estimating starch content, actual ethanol yield, and the relationship between these two values to further understand the conversion efficiency of sorghum grain among different varieties. This research was funded by the Center for Applied BioEnergy Research (CABER) and the Illinois Plant Breeding Center (IPBC) at the University of Illinois.
Many chemical changes in poultry meat occur during the first few hours postmortem. These chemical changes may affect the meat quality and therefore they affect the consumer. Rapid changes in the proteins and fats in meat begin immediately, coupled with the amount of time to prepare samples for analysis have rendered short time measurements very difficult to collect. Flash freezing the filets at set time points postmortem may be used to contain the chemical changes at a set point. Each filet was sectioned into four sections. Each of the four sections was aged less than one hour, 4 hours, 24 hours, and 120 hours before being flash frozen with liquid nitrogen, followed by freeze drying. The freeze dried samples were ground into a powder and visible-near infrared spectra were collected. Analysis of the spectra helped to identify changes in the proteins and fats of the breast filets. Additionally, peaks in the spectra from the freeze dried samples were able to yield information that had not been identified in previous attempts using raw samples due to the interference from water bands. The chemical changes identified in the meat samples during the rigor process may be used to help identify which mechanisms cause certain quality characteristics of the meat.
Agriculture

New Sorbent from Agro-Industrial Waste and Its Potential Use in 17 Beta-Estradiol and 17 Alpha-Ethynylestradiol Removal

In recent years, the increasing introduction of new chemicals in the market, and the development of more accurate analytical methods, added a variety of endocrine disruptors compounds (EDC) [1]. Even though they are found in very low concentrations (range of ng L-1) there is still a lack of knowledge about long-term risks of EDC for non-target organisms as well as for human health [2]. Usually, the current methods are not adequate for direct determination of target compounds at low concentration level in complex matrix samples. There is a need for developing new reliable analytical methods, which will enable a rapid, sensitive and selective determination of EDC in environmental samples. Therefore, a sample pretreatment step prior to chromatographic analysis is necessary for pre-concentrating the target analytes [3]. In order to solve this problem, there is a demand for new sorbents for solid-phase extraction (SPE) which may improve analyte recovery, sorptive capacity and selectivity. In this context, this study aimed to develop a new SPE sorbent from agroindustrial waste for removal of 17beta-estradiol (E2) and 17alpha-ethynylestradiol (EE2) from aqueous solution. The adsorption kinetic for E2 and EE2 in the first hours reached 95% of removal for both hormones (Figure 1). All experiments showed the potential of the agroindustrial sorbent as a new material for SPE devices.


Keywords: Absorption, Chemical, Environmental/Water, Fluorescence
Application Code: Environmental
Methodology Code: Fluorescence/Luminescence
Modern food industry heavily rely on chemicals to prevent and control stored product insect pests and assure food hygiene and security for worldwide trading. Fumigation process is therefore necessary to disinfect commodities stored in large quantity. For this purpose mainly phosphine and methyl bromide are used on large scale as fumigants, with different food exposure conditions and suitable quarantine period to minimize residues amount. In this respect, safety concerns strongly demand for high quality food contaminants free, especially pesticides and insecticides residues. This paper is focused on the optimization of a GC-TOFMS method for unequivocal quantitative determination of fumigant residues in different type of fresh food and grains as fresh fruit and rice, with more attention to the extraction technique, aiming to reduce the sample preparation step. The method was shown to be fast, free of interference and complying to the CODEX MRL guideline.
There is increasing demand to expand the scope of analytical methods employing GC/MS for food contaminants to include many hundreds of analytes. Although many laboratories have migrated their multi-residue pesticide methods from GC-MS to GC-MS/MS, as analysis suites become ever larger, concerns inevitably arise over instrument duty cycle. Another approach is to use a GC-Q/TOF system in full spectrum acquisition mode and to use the increased selectivity provided by high-resolution accurate mass measurements in order to measure, theoretically, an unlimited number of compounds. The additional sensitivity and selectivity provided by the GC-QTOF is particularly relevant for the analysis of pesticide residues in foodstuffs prepared by the QuEChERS sample preparation technique, where large amounts of sample matrix may be present in the final extract.

This presentation will introduce the All Ions workflow for the screening of pesticide residues in various foodstuffs using a GC-Q/TOF and electron impact ionization in combination with a retention time locked GC method, backflush for increased method robustness and a new accurate mass spectral database of pesticides.

The All Ions MS workflow for accurate mass GC/Q-TOF is seamlessly integrated into the Agilent Mass Hunter Qualitative software program. This new, comprehensive workflow for GC/MS accurate mass data uses Agilent’s proprietary Personal Compound Database and Libraries (PCDLs) to identify compounds using their precursor (reference) and fragment ions. The workflow includes the use of a new accurate mass PCDL that contains more than 700 pesticides and begins by looking for molecular evidence by isotope pattern matching, followed by examination of the co-elution profiles of fragment ions of target compounds.

Keywords: Gas Chromatography/Mass Spectrometry, GC-MS, Identification, Pesticides
Application Code: Agriculture
Methodology Code: Gas Chromatography/Mass Spectrometry
The traditional approach to analysis of metabolites by single quadrupole GC/MS requires that the samples be derivatized and analyzed within 24 hours to prevent degradation. With run times of 45 minutes or longer on conventional capillary columns, the number of samples that can be analyzed each day using this approach is limited. Another significant drawback to single quadrupole GC/MS is that medium-bore columns do not usually have sufficient resolving power to separate the target metabolites from the background matrix, making unambiguous compound identification and quantitation problematic. The shorter, narrow-bore columns are similarly not an option with GC/MS, as they have limited loading capacity which prevents trace-level detection required by metabolomics assays.

This poster presents a novel solution, using two dissimilar columns joined in series for chromatographic separation, with a triple-quadrupole GC/MS/MS operated in the Multiple Reaction Monitoring (MRM) mode for selective identification and trace-level quantitation of metabolites in serum. Two capillary columns were connected in series: a short column with 0.5-µm film thickness was used to provide the loading capacity, followed by a narrow-bore 0.15-mm ID column for chromatographic resolution of the 25 metabolites of interest in under 3 minutes (compared to 45 minutes on a conventional single column). Analysis by triple quadrupole GC/MS/MS in MRM mode removed any remaining background interference, and provided selective detection of the targets at concentrations in the sub-µg/mL (ppm) range. Instrument operating conditions and MRM transitions for 25 metabolites are described. Chromatographic separation in 3 minutes, calibration results, and repeatability statistics are presented. The improvement in analyte selectivity using MRM mode, compared to GC/MS is also illustrated.

**Keywords:** Bioanalytical, Gas Chromatography/Mass Spectrometry, Metabolomics, Metabonomics

**Application Code:** Clinical/Toxicology

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Gabapentin and pregabalin, a novel class of antiepileptic drug (AED), are widely used for the treatment of neuropathic pain and seizures. However, cases about misuse and abuse of these two AEDs have been often reported recently. For the purposes of compliance monitoring and drug abuse prevention, an accurate, sensitive and high throughput analysis method is highly demanded. Oral fluid, as an alternative of urine for drug testing, has significant advantages since its collection is efficient, non-invasive and readily observable. To the best of our knowledge, there is no published study of the use of oral fluid for monitoring of gabapentin and pregabalin in pain patients. The goal of this study is to develop and validate a high-throughput, sensitive and specific method for the quantitative analysis of gabapentin and pregabalin in oral fluid using HPLC/MS/MS system. Oral fluid samples were prepared by protein precipitation with acetonitrile containing internal standards and followed by dilution with water. Phenomenex C18, 2x50 mm 3.0 [micro]m HPLC column was used for the separation. HPLC/MS/MS experiments were performed using Shimadzu LCMS-8040 system. For quantification, the ion transitions monitored were m/z 160.20 -->55.05 and 160.20 -->55.05 for pregabalin and m/z 172.20 -->154.10 and 172.20 -->55.00 for gabapentin in multiple reaction monitoring (MRM) mode. The linear dynamic ranges for both drugs were 4-800 ng/mL with r2 >0.995. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the signal to noise ratio for the two drugs. The method was validated and the accuracy, precision and inter-day repeatability were all within the required limits. This method was also successfully applied to the compliance monitoring in pain patients.

Keywords: Clinical/Toxicology, Liquid Chromatography/Mass Spectroscopy
Application Code: Clinical/Toxicology
Methodology Code: Liquid Chromatography/Mass Spectrometry
An Evaluation of Biphenyl Chemistry to Aid in High-Throughput Bioanalytical LC-MS/MS Analyses

LC-MS/MS has become commonplace in the bioanalytical laboratory, as it can produce high throughput, high data quality analyses. The use of fully porous UHPLC and superficially porous HPLC columns are often used to increase the efficiency and peak capacity, and to decrease the analysis times. While these column advancements do impact efficiency, they do not directly impact selectivity or retention, which are the prominent parameters to analyte resolution.

In our experiments, we investigate a biphenyl based column chemistry to determine the effect on sample throughput and data quality in bioanalytical separations. From these applications we hope to demonstrate the advantages of the biphenyl-based stationary phase on throughput, data quality, sensitivity, and compound resolution.

Keywords: Clinical/Toxicology, HPLC, HPLC Columns, Bioanalytical
Application Code: Clinical/Toxicology
Methodology Code: Liquid Chromatography/Mass Spectrometry
Analysis of pain management and illicit drugs in oral fluid has attracted much attention in clinical toxicology recently. Oral fluid, as a promising test matrix, has significant advantages over blood and urine in terms of easy, rapid, non-invasive and observed specimen collection. However, storage and shipping of oral fluid have been proved challenging. Inspired by dried blood spots (DBS) technique, we hereby propose a dried spots technique for quantitative determination of pain management drugs and drugs of abuse in human oral fluid using liquid chromatography-tandem mass spectrometry (LC/MS/MS). Fifteen most commonly seen drugs, such as morphine, oxycodone, hydrocodone, tramadol, amphetamine, alprazolam, diazepam, THC, cocaine and etc. were spiked into drug-free saliva. Aliquot of 30 [micro]L spiked saliva was spotted onto Whatman filter paper and allowed to dry at room temperature. Two 6 mm disks were punched out of the filter paper and extracted by organic solvent containing internal standards. The extractant was then filtered before LC/MS/MS analysis. A 50x2 mm, 3 [micro]m C18 column was used for the high-throughput simultaneous separation of all 15 drugs. Quantitation was performed on a Shimadzu 8040 triple quadrupole mass spectrometer. Accuracy, precision, lower limit of quantification, linearity, and recovery of the method were evaluated. Most of the tested drugs were found to be stable in the dried spot for at least 30 days. The results indicate that dried spots technique is a promising specimen storage method for oral fluid drug testing. The authors gratefully acknowledge GE Healthcare for providing DBS supplies.
The chemical analysis of a sample of herbal product from Somali is reported. The herbal product is utilized as a topical anti-infective rub for wound treatment. This herbal product was attributed to Commiphora molmol species (Somalian myrrh) from Africa. An elaborate protocol for determining the components of herbal remedy was developed to collect the volatile and non-volatile components. The volatile components were extracted using steam distillation and the extract analyzed using GC-MS. The non-volatile components were extracted via soxhlet extraction using ethyl acetate and purified using column chromatography the non-volatile fractions are analyzed using GC-MS. The extracts yielded a wide range of components that were identified by mass spectral comparison with a built in mass spectral library in the GC-MS. Some of these compounds include: \(\gamma\)-elemene, \(\alpha\)-elemene, \(\delta\)-elemene, \(\alpha\)-cubebene, \(\beta\)-bourhonene, isogermafurene, humulene, \(\beta\)-ylangene, \(\alpha\)-copaene, elemol, cadinol.

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<th>Keywords:</th>
<th>Flavor/Essential Oil, Gas Chromatography, Gas Chromatography/Mass Spectrometry, GC-MS</th>
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Clinical Chemistry and Toxicology

Simultaneous Determination of 17 Drugs of Abuse and Organophosphorus Pesticides in Human Blood by GPC/GC/MS

On-line gel permeation chromatography/gas chromatography/mass spectrometry (GPC/GC/MS) is a unique technique to cleanup sample that reduce time of sample preparation. GPC can efficiently separate large molecules like fats, protein and pigments from target small molecules. Due to this advantage, GPC/GC/MS instruments are widely used for pesticide analysis. In this work, a new method was developed for rapid determination of 17 drugs of abuse and organophosphorus pesticides in human blood by GPC/GC/MS. The modified QuEChERS method was used for sample preparation. Blood samples (1 mL) were extracted using acetonitrile, MgSO4 and PSA powder. Finally, concentrated samples in acetone/cyclohexane (3/7, v/v) were obtained for GPC/GC/MS analysis. The correlation coefficients of calibration curves from 0.1 to 1.0 µg/L were all above 0.998. Most of analytes were measured at 0.01 mg/L level in a 1 mL human blood. The mixed standard solution was added into the blank blood sample to make the concentration of 0.1 and 1.0 µg/mL. For all of analytes, recoveries in the acceptable range of 70~115% and repeatability better than 5% (%RSD, n=3) were achieved for both matrices at spiking levels of 0.1 and 1.0 µg/mL. The performance of the method was satisfactory with results meeting validation criteria. The method is promising for biological analysis.

Keywords: Forensics, GC-MS, Pesticides, Toxicology

Application Code: Clinical/Toxicology

Methodology Code: Mass Spectrometry
Clinical Chemistry and Toxicology

Enhanced Resolution and Matrix Interference Reduction for the Analysis of Vitamin D Metabolites

Analysis of Vitamin D metabolites has continued to be a topic of interest in recent publications, primarily as biomarkers for possible disease states and vitamin sufficiency. While Vitamin D is present in two forms, current ELISA methods cannot distinguish D2 and D3 forms of the vitamin metabolites resulting in a reporting of total 25-hydroxyvitamin D. In this study, an LC/MS method for the analysis of Vitamin D metabolites is expanded to include dihydroxy metabolites along with the epi-homologs. Chromatographic resolution is utilized for the quantitation of hydroxy and dihydroxy Vitamin D2 and D3 metabolites including the isobaric epimers. In addition, sample preparation techniques are evaluated for the impact of biological matrix ionization effects.

Chromatographic method development consisted of screening C18, Cyano, Phenyl Hexyl and pentyl fluorophenyl (F5) stationary phase. Method development experiments resulted in conditions for the direct quantitation of isobaric metabolites 25 hydroxyvitamin D3, 3-epi-25 hydroxyvitamin D3 1-25 hydroxyvitamin D3 along with 25 hydroxyvitamin D2, 3-epi-25 hydroxyvitamin D2. In addition, human serum samples were processed using standard protein precipitation techniques along with novel phospholipid depletion plates for the comparison of matrix interference impact. The unique combination of the selectivity of the F5 separation along with the novel sample preparation technique allow of a robust and accurate LC/MS method for quantitation of all the associated Vitamin D metabolites.

Keywords: Clinical/Toxicology, HPLC Columns, Liquid Chromatography, Sample Preparation
Application Code: Clinical/Toxicology
Methodology Code: Liquid Chromatography
Selectivity is among the key requirements for ion-selective electrodes to be used reliably to detect the ions of interest in target samples. While there are many highly selective polymer membrane-based cation-selective electrodes, there are still limitations to design sufficiently selective membrane electrodes for anions. Thus, anion-selective electrodes, typically, exhibit large responses toward lipophilic anions. This possesses limitation on the use of anion-selective electrodes for the detection of hydrophilic anions such as total carbon dioxide (carbonate) and chloride in clinical samples due to interference from lipophilic ions such as salicylate and thiocyanate. It has been shown recently that such interferences can be alleviated by kinetic control of ion-selective electrodes under pulsed chronopotentiometric measuring mode. Here, a current pulse is applied across the membrane to cause ion flux from the sample to the membrane. Since the ion extraction is a diffusion-controlled process, depletion of dilute interfering ions occurs at the membrane surface and thus the measured potential will be essentially a function of the concentration of the relatively abundant hydrophilic target ions. It was shown also that cellulose triacetate (CTA)-based asymmetric membranes prepared by first casting a thin layer of CTA without carrier, hydrolyzing this layer with base, and then casting a second layer of CTA containing membrane active components on the side which was not hydrolyzed, have slow response kinetics toward lipophilic anions. This has been attributed to small hole-size of the hydrolyzed layer and the hydrophilicity due to hydrolysis. Thus, only low concentration of the lipophilic ions will diffuse to the inner sensing layer and this will be depleted by extraction into the membrane under pulsed chronopotentiometry. Here, we introduce this conjunction of asymmetric membrane and pulsed chronopotentiometry to enhance selectivity for chloride and carbonate ions.
Several years ago a novel working electrode material boron-doped diamond (BDD) became available, extending the range of compounds that could be measured using electrochemical detection. Although many different applications are now reported in literature, few have focused on clinical diagnostics. One example, however, is the routine measurement of the cellular antioxidant glutathione (GSH) and its disulfide (GSSG). Oxidative stress is thought to be associated with many diseases. Changes in the GSH/GSSG ratio are currently used as an indicator of the level of oxidative stress. Although there are numerous approaches to measure tissue levels of GSH and GSSG, many suffer from methodological issues including specificity and may even cause an artificial change in the GSH/GSSG ratio being measured. Other HPLC-based approaches with electrochemical detection enable direct, selective and sensitive measurement of GSH and GSSG. Unfortunately, methods using glassy carbon working electrodes are limited by the oxidation potential used (adversely affecting the sensitivity of GSSG) and require routine maintenance due to adsorption problems. Both of these problems are overcome with the BDD working electrode. Presented here is a fast and easy way for determining the level of oxidative stress using UHPLC with BDD electrochemical detection.

Another clinically relevant compound is the sterol, cholesterol, which is a major component of cell membranes and is associated with arteriosclerosis. Although simple kits are available for cholesterol measurement in plasma or serum, these do not provide any differentiation from phytosterols that may be present in the sample. A simple UHPLC method with electrochemical detection using the BDD working electrode is presented for the measurement of cholesterol in blood with minimal sample preparation. This method is sensitive (<100 pg/mL) and provides sufficient resolution so that the presence of phytosterols can also be determined.

Keywords: Bioanalytical, Clinical/Toxicology, Electrochemistry, Liquid Chromatography
Application Code: Clinical/Toxicology
Methodology Code: Liquid Chromatography
Clinical Chemistry and Toxicology

Target and Non-target Analysis of Metabolites in Urine Using Scan/MRM and GC/MS/MS

The conventional approach to analysis of metabolites using triple quadrupole GC/MS/MS typically involves two phases. In the discovery, or non-target phase, full-scan analysis is used to detect and identify the potential biomarker candidates. Acquisition of full scan spectral data can identify numerous individual metabolites through library matching, but there are often close- or co-eluting compounds that can hinder quantitation accuracy. The second phase, validation, is done in the Multiple Reaction Monitoring (MRM) mode, which provides selective confirmation of the individual biomarkers, as well as sensitive trace-level quantitation of the targeted metabolites.

For comprehensive metabolite analysis, both target and non-target methods must be used, but this approach requires multiple injections, and in many cases the amount of sample available for analysis is limited. Sample analysis in the simultaneous Scan/MRM acquisition mode provides identification of the non-targeted metabolites, while simultaneously validating and quantifying the targeted compounds. Using this approach, metabolomics discovery and validation can be included in a single analysis.

This poster will describe simultaneous Scan/MRM analysis mode as it applies to analysis of metabolites, and provide examples of how this approach was used to identify and quantitate individual biomarkers in urine samples. The results demonstrate that the Scan/MRM mode is an effective tool for tentative detection of non-target metabolites (Scan), and selective determination of target metabolites (MRM) using a single analysis.

Bioanalytical, Gas Chromatography/Mass Spectrometry, Metabolomics, Metabonomics, Tandem Mass Spectrometry

Clinical/Toxicology

Gas Chromatography/Mass Spectrometry
Ethylsulfate (EtS) and ethylglucuronide (EtG) are metabolites of ethanol, commonly monitored in forensic toxicology applications as evidence of alcohol consumption. Sample preparation techniques for the analysis of these compounds in biological matrices can run from simple dilute-and-shoot through complex solid phase extraction. The purpose of this study was to compare these sample preparation options in the context of not only the analytical result, i.e. being able to achieve the required detection limits, linearity, and precision, but also in evaluating other factors that are important aspects of selecting the technique to use. Protein precipitation, mechanical filtration, “hybrid” filtration, and solid phase extraction were compared, with liquid chromatography and tandem mass spectrometry (LC/MS/MS) used as the technique for assessing extraction performance and efficiency. The advantages and disadvantages of each approach will be presented, with a discussion about the relative cleanliness of each technique as determined using LC-QTOF for high-resolution evaluation of the samples. Comparing the methods based on factors above and beyond the method performance allows decisions to be taken that best suit the laboratory needs for generating defensible, accurate data while also maximizing uptime and efficiency.

**Abstract Text**

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**Keywords:** Bioanalytical, Biological Samples, Forensic Chemistry, Sample Preparation

**Application Code:** Clinical/Toxicology

**Methodology Code:** Sampling and Sample Preparation
Breath gas analysis is a non-invasive approach to achieve the medical condition of an individual. By detecting and quantifying the concentration of certain markers it is possible to diagnose a variety of illnesses. Another feature of breath air analysis is to gain information about the vital status by monitoring the lung function. A multi sensor system for breath air analysis has been developed at the Institute of Measurement Technology. The system is built as a handheld device containing five different sensors. A differential pressure sensor allows the determination of the lung volume and the stamina of the test person. A semiconductor gas detector is used to measure the ethanol concentration. O2 concentration is detected by using an electrochemical cell. The system also allows the quantification of CO2 and acetone with the help of a NDIR sensor and a PID. The casing of the sensor system was designed using 3D-CAD software and produced with rapid prototyping techniques. To control the system an iPad mini was fitted onto the casing. It is connected to the sensor system by establishing a WiFi connection. The iPad mini software allows the user to monitor the response of each sensor and shows the concentration of every measured substance and the lung volume online. The system setup will be presented.
Determination of Blood Alcohol Content (BAC) has been a standard analytical method in criminal labs for many years. The typical instrument configuration consists of a static headspace instrument for sample introduction, followed by gas chromatography (GC) with two dissimilar capillary columns for separation, and two Flame Ionization Detectors (FIDs) for detection and quantitation. Two sets of data are obtained simultaneously, and the quantitative results from the two FIDs are compared for confirmation of the reported BAC levels.

With the BAC method, compound identification is done by comparing the retention time (RT) of blood alcohol in the unknown sample to the RT obtained from analysis of an analytical standard. Recently however, additional compound identification provided by matching the alcohol mass spectrum to a library spectrum, in addition to RT, has proven to offer an additional level of confirmation.

This poster describes BAC analysis using GC-FID in parallel with a mass spectrometer (MS) for positive compound identification. Instrument configuration, optimized analytical conditions, and full analytical results for both detectors are presented.

**Keywords:** Forensics, Gas Chromatography/Mass Spectrometry, Headspace, Toxicology

**Application Code:** Clinical/Toxicology

**Methodology Code:** Gas Chromatography/Mass Spectrometry
In traditional medicine, mulberry (MLE) and jackfruit leaves extracts (JLE) were used for the treatment of diabetes mellitus. The aim of this study was to evaluate the comparative efficacy of either (MLE) or (JLE) alone or in combination and gliclazide on streptozotocin/nicotinamide (STZ/NA)-induced diabetes in rats for 8 weeks. Diabetes induced in rats by a single intraperitoneal injection of STZ (65 mg/Kg). Results showed that diabetic rats had significant increase in plasma glucose, (HbA1C%), triglycerides, total cholesterol, LDL-C, TBARS, nitric oxide (NO) and liver tissues DNA damage levels compared to normal controls. In contrast, liver glycogen, plasma HDL-C, GSH levels and erythrocyte superoxide dismutase (SOD) and catalase (CAT) activities were significantly decreased. Plasma glucose, (HbA1C%), Liver glycogen levels were insignificantly decreased by treatment of diabetic rats with (MLE) or (JLE), and were normalized by gliclazide. In particular, the administration of (MLE) or (JLE) alone or in combination tends to bring the lipid profile parameters and (LDL/HDL) risk factors values of diabetic rats to near normal values. The alterations in plasma TBARS, GSH and (NO) levels were reverted to near normal with (D + MLE) and [D + (MLE + JLE)], respectively. Moreover, (MLE) or (JLE) markedly restored the activity of erythrocyte SOD, CAT to normal values. Finally, all treatments resulted in disappearance of DNA damage forms released from the diabetic rats. In conclusion, our findings suggest that (MLE) or (JLE) treatment exerts a therapeutic protective effect in diabetes by decreasing oxidative stress, hyperlipidemia and hepatic tissues DNA damage.

Acknowledgments
We thank the NODCAR (National Organization of Drug Control and Research), Cairo, Egypt for financial support in the form of a Major Research Grant.

Keywords: Biological Samples, Biomedical, Biopharmaceutical, Natural Products
Application Code: Biomedical
Methodology Code: Process Analytical Techniques
Development of methods for analysis of drugs of abuse has become a high priority for both toxicology and enforcement requirements. The large numbers of individual drugs and new “designer drugs” has made method development for these compounds particularly challenging.

Gas chromatography mass spectrometry (GC/MS) has been used extensively for analysis of drug residues and trace level drugs in biological fluids. One of the most significant challenges has been matrix interference and achievement of meaningful detection limits for the compounds of interest. Triple quadrupole GC/MS/MS operated in the Multiple Reaction Monitoring (MRM) mode has emerged as a powerful technique for trace level analysis in complex matrices.

Deuterium-labeled analogs of target drugs are widely used as internal standards and analytical method controls for precise drug assays. In some cases, the mass spectra of the deuterium-labeled analogs differ only slightly from the corresponding unlabeled compounds. Mass spectral similarity calls into question the specificity of GC/MS/MS operated in the MRM mode when co-eluting compounds produce common product ions. One example is the analysis of hydrocodone, a common opioid narcotic using hydrocodone-n-methyl-d3 as an internal standard.

The poster presents instrument configuration, operating parameters, and analytical results for analysis of three common opioids with hydrocodone-d3 as an internal standard using a triple quadrupole GC/MS/MS in the MRM mode.

**Keywords:** Clinical/Toxicology, Drugs, Gas Chromatography/Mass Spectrometry, Toxicology

**Application Code:** Clinical/Toxicology

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Clinical Chemistry and Toxicology

Matrix Specific Sample Preparation Strategies for Opioid Analysis

The analysis of natural and synthetic opioid drugs continues to be an important area of clinical research. Depending on the application, these drugs may be analyzed in urine, blood or, increasingly, oral fluid. These matrices each have individual challenges which need to be overcome. The methods presented herein describe strategies for sample preparation in each of these three matrices followed by reversed-phase UHPLC/MS/MS analysis. Using a silica-hybrid UHPLC column, all analytes eluted in less than 5.5 minutes, and even the most polar compounds were adequately retained. This allowed for direct analysis of glucuronide metabolites, eliminating the need for enzymatic hydrolysis.

Urine and oral fluid samples were both prepared using mixed-mode strong cation exchange solid phase extraction (SPE). Whole blood samples were prepared using phospholipid removal plates which allowed for cell lysis and protein precipitation within a single well, followed by vacuum elution into a 96-well collection plate. For urine samples, mixed-mode SPE prepared samples demonstrated reduced matrix effects, and improved sensitivity, linearity, accuracy and precision compared to samples prepared by a simple dilution protocol. Oral fluid samples prepared by mixed-mode SPE also had excellent linearity and quantitative performance. In addition, the Elution format was ideal for the limited sample volumes often encountered with oral fluid samples. The phospholipid removal plates used for whole blood processing enabled rapid cleanup and processing with minimal need for method development. This represents a comprehensive sample preparation and analysis strategy for this important group of compounds in three important matrices.

Disclaimer: This method is intended for clinical research use only, not for use in diagnostic procedures

Keywords: Clinical Chemistry, Liquid Chromatography/Mass Spectroscopy, Sample Preparation, Solid Phase Extr
Application Code: Clinical/Toxicology
Methodology Code: Liquid Chromatography/Mass Spectrometry
Aspartame (N-L-aspartyl-L-phenylalanine methyl ester) is a low-calorie sweetener commonly used in carbonated soft drinks and beverages. In this study, a rapid and simple method was developed for the quantification of aspartame in food samples using surface enhanced Raman spectroscopy (SERS). Silver nanoparticles (Ag-NPs) were synthesized by wet chemistry method and characterized with UV-Visible spectrophotometer and TEM. Developed method was based on the enhancement of aspartame Raman signal in the presence of Ag-NPs. Aspartame solutions between 0.2 mg/ml and 1.2 mg/ml were prepared for Raman measurements. The calibration curve was obtained by plotting Raman band intensity against aspartame concentration. A linear relationship was obtained with a high determination coefficient value ($R^2 0.96$). Real sample analyze was performed successfully using developed method. Short analyze time (20 seconds), small sample size requirement and performing aspartame analyze without pretreatment were brought to this method more practical and applicable to field analysis with portable Raman systems.

Keywords: Food Science, Surface Enhanced Raman
Application Code: Food Science
Methodology Code: Biospectroscopy
Fluorinated grease proofing agents are used as food contact materials. Previous studies have shown these compounds can migrate into the food, providing a source of exposure. If migration occurs, these ingested compounds might be absorbed, degraded or transformed in the gastrointestinal tract. The fate of ingested fluorinated grease proofing agents is unknown. Use of [i]in vitro[/i] gastrointestinal digestion can model the availability of fluorinated grease proofing agents for absorption from the digested food matrix.

The [i]in vitro[/i] digestion system used here was a two-step process of simulated gastric digestion followed by simulated intestinal digestion and diffusion across a dialysis membrane. The gastrointestinal digestion was used to evaluate the availability of technical fluorinated grease proofer formulations used as food contact materials. Gastrointestinal digestion with large molecular weight grease proofing agents is difficult, in part because industrial standards of these formulations are complex. Previous studies have shown that fluorinated grease proofers can act as environmental precursors to perfluorocarboxylates (e.g. perfluorooctanoic acid). These smaller derivatives, which are available in higher purities, can be used analytically (mass balance, LC-MS/MS) to determine the parameters of gastrointestinal digestion.

A method for the determination of the availability and diffusion of commercial grease proofing agents using gastrointestinal digestion has been developed. Method validation and optimization of post digestion clean-up will be presented. Preliminary results indicate that a commercial perfluoroalkylphosphate has an in vitro availability of about 0.3% while perfluorooctanoate has an availability of about 4%.

Keywords: Food Science, Membrane, Method Development
Application Code: Food Science
Methodology Code: Liquid Chromatography/Mass Spectrometry
Many tropical plants are important sources for biologically active compounds that have potential therapeutic effects. 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, and chronic ulcer. Recently mangosteen has been proposed 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.

The method we have developed for analysis of xanthones in mangosteen pericarp combines accelerated solvent extraction (ASE) and UHPLC separation. Conventional extraction methods for mangosteen paricarp, such as soxlet, are time consuming, labor intensive and do not always deliver the desired reproducibility. The ASE system has the advantages of short extraction time, low solvent consumption, high extraction efficiency and excellent reproducibility. The ASE extraction described in this poster requires only 30 mins and delivers $\geq 95\%$ recovery with excellent reproducibility.

The UHPLC method for xanthone analysis employs a Dionex Acclaim RSLC C18 column with UV detection and is completed in 25mins. Comparative data using charged aerosol detection and electrochemical detection are also discussed. Five major xanthones, including $\alpha$-mangostin, 3-isomangostin, gartanin, 9-hydroxycalabaxanthone, and 8-desoxygartanin, were quantitatively determined in mangosteen pericarp.
Chemical Tuning Method to Selective Enrichment of Vegetal Selenoproteins Using Synchrotron XANES Techniques

The present study develops a method to control the enrichment of plants and particularly wheat plants in organic selenium, particularly selenomethionine (SeMet), selenocysteine (SeCyst) or a mixture of both, when the plant is fed with a nutrient solution enriched with either sodium selenite, sodium selenate or a mixture of both salts at a controlled molar ratio that produces corresponding SeMet/SeCyst ratio, leading thus to a selective selenoaminoacid production by varying the indicated Selenite/Selenate molar ratio (chemical tunning), regulation that can be expressed in terms of the related redox potential of the nutritional solution to produce selective enriched plants in organic selenium using this method. Control of the produced selenoaminoacids and thus the above relationship has been carried out by direct speciation of Selenium using Synchrotron XANES techniques.

REFERENCES

The present study has been supported by the Spanish projects CTQ2009-07432 and CTM2012 30970

Keywords: Amino Acids, Biological Samples, Food Science, Speciation
Application Code: Food Science
Methodology Code: Other (Specify)
It’s demonstrated that the combination of benzoate salts with ascorbic acid produces low levels (ng/g) of benzene. Benzoates are widely used as food preservatives and especially beverages, like fruit-flavored soft drinks, suffer potentially for benzene formation. Benzene contamination was recently found also in benzoate free foods, especially in carrots products for infants. The chemical process that leads to benzene formation is still unclear: heat-treatments seem to have a key role, promoting the benzene release from suspected precursors (beta-carotene and terpenes). Due to benzene high carcinogenic activity, its presence in food and beverages is of great concern. In particular, contamination in baby food is even more critical, since infants are more exposed to toxicity than adults. The new generation of Static Headspace Autosampler coupled to FastGC-TOFMS system, is proposed for benzene contamination assessment in different food and beverages products. Besides a quick sample preparation offered by the static headspace technique, lower detection limit and outstanding repeatability are achieved. Fast GC allows to complete the chromatographic run in 10 minutes, maintaining the required separation power and improving the sample throughput. The use of Fast GC enhances the potential of High Speed TOF-MS technology, in which the acquisition rate can be increased without sacrificing sensitivity or losing spectral information. High quality mass spectra and effective deconvolution algorithm guarantee a reliable identification, even in case of co-elution and matrix interferences. The obtained results confirm that the described system offers the specificity and sensitivity required to detect benzene in different food and beverages samples.

Keywords: Beverage, Food Science, Gas Chromatography/Mass Spectrometry, Headspace
Application Code: Other (Specify)
Methodology Code: Gas Chromatography/Mass Spectrometry
Astaxanthin is a xanthophyll carotenoid found abundantly in [i]Haematococcus pluvialis[/i] algae. Due to its high antioxidant activity, it is widely used in food, cosmetics and dietary supplements. Currently, quantitative analysis of astaxanthin employs spectrophotometric or HPLC methods. The spectrophotometric method, however, often suffers from low accuracy; while the HPLC methods require the use of complex solvent systems and long analysis time due to the low polarity of the analyte. With a growing industry-wide focus on monitoring micro-nutrients in fortified foods and supplements for regulatory compliance, there is an increasing demand for rapid and reliable analytical methods for the quantitative analysis of astaxanthin.

Due to non-polar CO[sub]2[/sub] being the primary constituent of the mobile phase, UltraPerformance Convergence Chromatography [sup]TM[/sup] (UPC[sup]2[/sup]) becomes a logical choice for improving analysis time. In this poster, a systematic study on developing a fast and reliable UPC[sup]2[/sup] method for astaxanthin quantitation will be presented. With the proper choice of column, mobile phase, and temperature, a 2-min UPC[sup]2[/sup] method was developed for the quantitative analyses of different astaxanthin supplements. The UPC[sup]2[/sup] method was also compared to a HPLC method in efficiency, speed, accuracy and reproducibility. Our results suggest that UPC[sup]2[/sup] shows great promise in becoming a routine analytical technique for quantitative analysis of astaxanthin and fat-soluble carotenoids in general.

**Keywords:** Method Development, Natural Products, Quantitative, SFC

**Application Code:** Food Science

**Methodology Code:** Liquid Chromatography
The significance of the interaction between Levofloxacin and Brassica oleracea capitata var. Alba L. (Green Cabbage) a commonly eaten vegetable in Nigeria was evaluated in this study. The pharmacokinetic parameters of Levofloxacin administered orally to cabbage pre-treated male albino Wister rats was established. Blood samples from the rats were collected over 48h for the quantification of Levofloxacin using HPLC. The presence of green cabbage extract significantly caused a 1.3-fold increase in the AUC0- of Levofloxacin (285.15 to 359.81) but 3.6-fold decrease in Vd, volume of distribution (1.53 to 0.43) and two-fold decrease in F, bioavailability (90.2 to 49.5). Findings from the study suggested that co-administration of green cabbage with Levofloxacin may result in antagonistic interactions causing negative clinical implications with decreased distribution and bioavailability of Levofloxacin in the rat. The nutrients of green cabbage were also discovered to be Zn, Mn, Fe, Cu, Cr, Ca, K, Mg and Na using the atomic absorption spectroscopic analysis. Alkaloids, glycosides, saponins, flavonoids, steroids, terpenoids, phenols, amino acids, carbohydrates and tannins were found to be in varying degrees. Should the use of both agents be required, it can therefore be suggested that sufficient time (about 3hrs) should be allowed between their administrations to ensure therapeutic efficacy of Levofloxacin.

Keywords: Drugs, Food Science, HPLC, Pharmaceutical

Application Code: Food Science

Methodology Code: Liquid Chromatography
Coffee is a requirement for many people to start their day. However, the reason people drink coffee may be more for the caffeine than for the taste. In order to determine the amount of caffeine in coffee, many coffee producers use liquid-liquid extraction. How much caffeine is in your cup of coffee? Assorted coffee blends will be extracted for caffeine using an automated liquid-liquid extraction technique with the intention of answering this question.

Keywords: Extraction, Food Science, GC-MS, Sampling
Application Code: Food Science
Methodology Code: Sampling and Sample Preparation
On a daily basis, adults and students often turn to coffee and energy drinks to give them enough energy to take on the day. Two of the main active ingredients found in these beverages are caffeine and taurine. The daily recommended value of caffeine, a stimulant drug, is 400 milligrams. This can be a problem for avid coffee drinkers as a grande size Starbucks cup of coffee can contain over half of the daily recommended value. It has been shown to increase alertness, mood, and to counteract sleep deprivation, but has also been linked to nausea, insomnia, and severe headaches. Another active ingredient, taurine has a recommended intake range from 100 to 400 milligrams per day. Surprisingly, most energy drinks contain 1000 milligrams per serving, and some energy drink cans contain more than one serving. Taurine was found to be beneficial in stroke and cardiovascular disease prevention when taken in moderate doses. A study, which tested the patients ability to complete verbal, spatial, and object related tasks, found that taurine increased accuracy on choice response assignments, but lowered the response time on verbal related tasks and did not show any increase in performance in trials associated with psychomotor performances. With these main ingredients surrounding students and adults on a daily basis, this project aims to determine the amount of each ingredient in beverages, such as energy drinks and coffee, and see if these results can be replicated across various instrumentation such as HPLC, UV-VIS, and IR spectroscopy.

**Keywords:** Beverage, Food Science, Gas Chromatography/Mass Spectrometry, Liquid Chromatography

**Application Code:** Food Science

**Methodology Code:** Separation Sciences
Emulsifiers are used to maintain a uniform suspension of immiscible materials. These compounds are typically surfactants, and can be designed for use in specific applications and products. Acylglycerols are used in food products containing oil and water (e.g. margarine, mayonnaise); lecithin is commonly found in chocolate and spray oils; acid esters of monoglycerides are used as dough conditioners in the baking industry; and hydroxypropylmethyl cellulose (HPMC) is used to thicken dairy products and help improve flavor characteristics. HPMC is also an important emulsifier used in the pharmaceutical industry.

The analysis of emulsifiers is becoming increasingly important, for both purity and stability properties. High pressure liquid chromatography is one of the more prevalent methods for analyzing these compounds. However, the majority of analytes do not contain a chromophore, which then requires the use of a universal detector, such as evaporative light scattering, refractive index, or charged aerosol detection. The Corona charged aerosol detector was used in the analyses of different emulsifiers that were extracted from food products, and results are provided.

The Corona Veo charged aerosol detector, a sensitive mass-based detector, is ideally suited for the direct measurement of emulsifiers, as they are non-volatile and non-chromophoric compounds. It offers excellent sensitivity (down to low nanogram amounts on column), a dynamic range of over 4 orders of magnitude, and similar inter-analyte response independent of chemical structure.

Keywords: Food Science, HPLC, HPLC Detection, Pharmaceutical
Application Code: Food Science
Methodology Code: Liquid Chromatography
The recent development of fused-core technology in HPLC columns is enabling faster and highly efficient separations. This technology was evaluated for the development of a fast analysis method for -ecdysone in extracts of Pfaffia glomerata. A step-by-step strategy was used to optimize temperature (30-55 ºC), flow rate (1.0-2.0 mL min⁻¹), mobile phase composition and equilibration time (1-5 min). A gradient method has been developed using two solvents: 0.1% acetic acid in water and 0.1% acetic acid in acetonitrile. Optimized conditions provided a method for the separation of -ecdysone and an unidentified peak eluting near -ecdysone in approximately 2 min and total analysis time (sample-to-sample) of 9 min. Evaluation of chromatographic performance revealed excellent reproducibility intraday and interday, resolution, selectivity, peak symmetry and low limits of detection and quantification levels. The robustness of the method has also been calculated according to the concentration / dilution of the sample and the injection volume. The sample solvent has been evaluated, obtaining that the best solvent to employ is methanol 80% in water. This developed method has been compared to other methods existing in the bibliography, achieving shortens the analysis time using a HPLC methodology. Finally, the developed method was validated with different extracts of Pfaffia glomerata samples. Funding from FAPESP (Project 2013/04304-4) is acknowledged.
Food Science: Analytical Methods

Ultrasound-Assisted Extraction of Curcuminoids from Curcuma Longa

Turmeric (Curcuma longa L.) is a plant rich in curcuminoids, which are natural pigments widely used by the food industry and with potential to be used in the prevention and treatment of degenerative diseases due to their biological activity. The extraction of curcuminoids is usually performed using outdated and less efficient methods and techniques. Ultrasound-assisted extraction is among the new techniques available that can be explored to improve the extraction process. The enhancement of the extraction with ultrasound is attributed to the phenomenon of cavitation and disruption of sample cells due to mechanical stress. This study aimed to evaluate the extraction of curcuminoids with ethanol and assisted by ultrasound. The evaluated parameters influencing the process were the ultrasound output power (160W, 320W, 480W, 640W and 800W) and temperature of extraction medium (40, 50, 60 and 70 °C). Extraction time was fixed in 20 min. The use of ultrasound for the extraction process of curcuminoids provided high recovery even at low temperature (40 °C), reaching 3.9% yield using 640W of ultrasonic power. Although the highest yield of curcuminoids was achieved at 60 °C also with 640W of ultrasonic power (4.2%), differences was not statically significant. In general, as temperature and ultrasound power increased, recovery of curcuminoids followed the same trend until a certain point, where recovery either was lower or there was no significant difference. Results indicate that application of ultrasound can be an effective alternative to improve the extraction of curcuminoids and reduce the time required for sample preparation.

Abstract Text

Keywords: Extraction, Food Science, Natural Products, Sample Preparation

Application Code: Food Science

Methodology Code: Sampling and Sample Preparation
The determination of the composition in Fatty Acid Methyl Ester is a common and challenging analysis in food chemistry, since the products can contain a complex mixture of saturated, monounsaturated, and polyunsaturated fatty acids, each with a variety of carbon chain lengths. The objective of this study was to develop an optimized and rapid analytical method, compliant with EN ISO 5508 & 5509 standards, which would automate the sample preparation step and achieve correct identification of the FAMEs in different vegetal oils. The analysis was conducted with PR2100 gas chromatograph (Alpha MOS, France) equipped with a stabilwax column (30m, 0.53mm, 1[micro]m), a Flame Ionization Detector (FID) and a PAL RTC autosampler (CTC Analytics, Switzerland). The derivatization step aimed at neutralizing the polar carboxyl functional groups was fully automated with the autosampler. It consisted of adding NaOH/MeOH to 10[micro]L of oil and heating at 80[degree]C for 5 minutes. Then, BF3/MeOH were added and the mixture was heated for 2 minutes at 80[degree]C. Finally, iso-octane and a saturated NaCl solution were added. The total derivatization time was 720 seconds. By applying a temperature gradient to the column, a successful separation and identification of the C14:0 to the C24:1 FAMEs was achieved in 800 seconds. This whole method thus allowed to determine the FAMEs composition in about 25 minutes for the first sample. Then, the overlapping between analysis run and the preparation steps of the next samples enables to reach a high analysis throughput.

Keywords: Chromatography, Derivatization, Food Science, Gas Chromatography
Application Code: Food Science
Methodology Code: Gas Chromatography
Differentiation the origin of the meat species and meat products was successfully performed based on their extracted fat samples using Raman spectroscopy and the principal component analysis (PCA). Extracted fat samples which were obtained from different meat species (cattle, sheep, goat, buffalo, pig, fish, poultry (chicken and turkey)) and their salami products were assayed based on their Raman measurements of their fat profiles. The collected Raman data were analyzed with a four-stage PCA. Different combinations of pre-processing techniques (baseline correction, different orders of derivatives, mean centre, normalising, smoothing, and auto-scaling) were applied to Raman data before the creating of the models to enhance the performance and the decomposition ability of the model. Four different PCA models were created with a total of 132 Raman data of the samples. The principal component (PC) scores were used to plot the score graphs of the samples and to create the clusters of same meat species in the same area. Seven meat species and their salami products were successfully differentiated from each other according to their origin. The results showed that the method that is Raman spectroscopy with PCA is practicable and usable analysis technique for the determination the origins of meat species in a very short analysis time (30 s) without requiring complicated chromatographic, immunologic or genetic methods and instruments and also trained personnel.

**Keywords:** Chemometrics, Food Science, Quality Control, Raman

**Application Code:** Food Science

**Methodology Code:** Chemometrics
Voltammetric determination of lactose using catalyst modified electrode is discussed in the presentation. The voltammogram shows two oxidative peaks for lactose in a pH 10 phosphate buffer solution. The detection limit for lactose is determined in milligram range. Optimization, interference effects, and stability studies will be discussed.
Spectro-eletrochemical experiments can provide \textit{in situ} characterization of electron transfer reactions. The combination of different electrochemical techniques, such as amperometry and cyclic voltammetry, with Raman spectroscopy, can monitor how molecules behave at the electrode surface. Our research uses surface-enhanced Raman spectroscopy (SERS) for the \textit{in situ} monitoring and characterization of molecules on microelectrodes in microfluidic devices. These devices are prepared utilizing methodology that produces a highly sensitive and enhancing polystyrene-encapsulated electrode and fluidic tubing. Multiple electrode materials can be embedded into the polystyrene base, and different metal combinations are deposited onto the wires, such as silver or gold, to form the SERS active microelectrode. The addition of a PDMS microchannel allows for flow on top of the electrode. This set-up is being used to investigate the electron-transfer of different analytes in the steady-state, as well as in flow.

**Keywords:** Electrochemistry, Electrode Surfaces, Spectroelectrochemistry, Surface Enhanced Raman

**Application Code:** Bioanalytical

**Methodology Code:** Molecular Spectroscopy
With single-molecule spectroscopy and confocal microscopy, we report a comprehensive analytical method for probing molecular transport in nanopores of silica in the laser probe volume at single-molecule level. The method provides time resolution for microsecond kinetic processes, such as diffusion, spatial resolution for locating diffusing molecules into ellipsoidal shells of nanometer thickness, and molecular resolution to record the distributions of physicochemical properties of the nanopores.

The following investigations have been performed: Molecules diffusing through the probe volume emit bursts of photons. Clustering coefficient of photon bursts signifies the random arrival of single molecules across the probe volume. The burst heights scale linearly with the excitation power but not with molecular concentration. Instead, the molecular concentration is analyzed through single molecule counting. The noise in single molecule counting follows shot noise distribution that is reduced when more molecules are counted. The strengths of the photon bursts correlate with the trajectory of molecules with the ones diffusing closer to the center of the Gaussian beam emitting stronger bursts while ones crossing the peripheral of the beam emitting weaker bursts. Probability distribution functions are derived for the localization of molecules into super-resolution ellipsoidal shells that exhibit equal photon counts. Diffusing coefficients are evaluated through the autocorrelation function. Single molecule analysis has shown that the nanostructures in the particle are uniformly distributed within the probe volume of ~ 400 nm and that there are no large obstacles in the molecular diffusion. There is a Gaussian distributed population of diffusion coefficients inside the nanopores, indicating a distribution of pore sizes and/or pore architecture.

We acknowledge the National Science Foundation (NSF) as the source of funding.

**Abstract Text**

**Keywords:** Fluorescence, Method Development, Microscopy, Spectroscopy

**Application Code:** Nanotechnology

**Methodology Code:** Molecular 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 and higher operating temperatures in the detector. 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 because of their simple manufacture, have a much higher vapor pressure and have 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 technology is not free of false alarms. This is why often different detection technologies are 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 which can be achieved (similar to IMS). In Raman spectroscopy the identification of explosives is based on their molecular vibrations, which is an orthogonal technique to the IMS. A new combination of IMS and SERS in one instrument for the simultaneous detection of all kind of explosives will be discussed. The improvements, compared to a standard IMS system will be shown.
The infrared and Raman spectra (3200 to 50 cm\(^{-1}\)) of the gas, liquid or solution, and solid have been recorded of isocyanocyclopentane, C-C\(_{5}\)H\(_{9}\)NC. FT-microwave studies have also been carried out and 23 transitions were recorded for the envelope-axial (Ax) conformer. Variable temperature (-65 to -100\(^\circ\)C) studies of the infrared spectra (3200 to 400 cm\(^{-1}\)) dissolved in liquid xenon have been carried out. From these data, both the Ax and envelope-equatorial (Eq) conformers have been identified and their relative stabilities obtained. The enthalpy difference has been determined to be 102 ± 10 cm\(^{-1}\)(1.21 ± 0.11 kJ mol\(^{-1}\)) with the Ax conformer the more stable form. The conformational stabilities have been predicted from ab initio calculations by utilizing several different basis sets up to aug-cc-pVTZ from both MP2(full) and density functional theory calculations by the B3LYP method. Vibrational assignments have been made for the observed bands for both conformers with initial predictions by MP2(full)/6-31G(d) ab initio calculations to obtain harmonic force constants, wavenumbers, infrared intensities, Raman activities and depolarization ratios for both conformers. The structural parameter values for the Ax form are; for the heavy atom distances (Å): C\(_{N}\) = 1.176 (3); C\(_{C}\)-N = 1.432 (3); C\(_{C}\)-C\(_{C}\)-C\(_{C}\) = 1.534 (3); C\(_{C}\)-C\(_{C}\)-C\(_{C}\)-C\(_{C}\) = 1.542 (3); C\(_{C}\)-C\(_{C}\)-C\(_{C}\)-C\(_{C}\) = 1.554 (3) and angles ('\(\phi\)) uplift(C\(_{N}\))= 177.8 (5); uplift(C\(_{N}\))= 102.9 (5); uplift(C\(_{N}\))= 103.6 (5); uplift(C\(_{N}\))= 105.9 (5). The results are discussed and compared to the corresponding properties of some related molecules.

**Keywords:** Raman, Spectroscopy, Vibrational Spectroscopy

**Application Code:** General Interest

**Methodology Code:** Vibrational Spectroscopy
Crude oil fouling in heat exchanges is one of the most challenging problems in petroleum refinery. The fundamentals of the complex fouling process are not fully understood which leads to inefficiency of preventing of depositions in heat exchanges. Asphaltenes has been closely linked and related to levels of fouling in heat exchangers. ATR-FTIR spectroscopic imaging is a powerful tool for providing visualisation and chemical analysis when applied for studying various dynamic systems and samples. Here we report on application of ATR-FTIR spectroscopic imaging for characterisation of asphaltenes and for in situ visualisation and chemical characterisation of the deposits precipitation from crude oil.

ATR-FTIR spectroscopic approach was applied for studying fouling processes via in situ chemical imaging of deposits precipitation from crude oil under compositional changes. The dynamics of deposits precipitation induced by n-heptane has been monitored starting from the formation of small particles on the measuring surface of the ATR crystal followed by their growth and aggregation. Deposits formed have been chemically characterised using extracted ATR-FTIR spectra. This study has demonstrated the feasibility and potential applications of ATR-FTIR spectroscopic imaging to understand how molecular and chemical structures of the deposits relate to components of the crude oil. This information about asphaltene deposits will provide new insight and understanding of the fouling process.

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**Keywords:** FTIR, Fuels\Energy\Petrochemical, Hydrocarbons, Imaging

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

**Methodology Code:** Molecular Spectroscopy
Levofloxacin is a fluoroquinolone antibacterial agent. Fluoroquinolones are not isolated from micro-organisms but synthesized by chemists. They are very active antibacterial agents with broad spectrum of activity.

The solid state interaction between levofloxacin and some metal cations; Zn2+, Al3+ and Cu2+ was carried out and the site of complex formation was attested to by Fourier Transform-Infrared (FT-IR) spectrophotometric spectra obtained.

The FT-IR spectrum of the levofloxacin alone showed very conspicuous absorption band at 1724.1 cm⁻¹, which totally disappeared on the levofloxacin – metal complex spectrum.

In this study it was observed that the levofloxacin formed complexes with Zn2+, Al3+ and Cu2+ (divalent and trivalent) metal cations.

Levofloxacin reacted with Zn2+, Al3+ and Cu2+ metal ions and we suggest that the complexation is on the carbonyl functional group due to the disappearance of the carbonyl band on the spectra of the levofloxacin-metal complexes.

Acknowledgement
We acknowledge the department of Chemistry, Faculty of Science, University of Lagos, Akoka campus, for allowing the use of the Fourier Transform-Infrared (FT-IR) spectrophotometer.

Keywords: Analysis, FTIR, Near Infrared, Spectroscopy
Application Code: Process Analytical Chemistry
Methodology Code: Near Infrared
Surface-Enhanced Raman Spectroscopy Platforms for Studying Electrodeposition and Surface Chemistry of Nanostructured Semiconductors

This presentation will highlight data that describe an overlayer surface-enhanced Raman spectroscopy (SERS) strategy for studying crystal quality of thin film semiconductors and the interfacial bonding with foreign adsorbates. The study was motivated by the significant influence on semiconductor performance in energy conversion, sensing and microelectronics by material crystallinity as well as interfacial chemistry. In this work, Raman data detailing phonon evolution of Cd-based II-VI semiconductor at Au nanoparticle substrate during electrodeposition will be presented to first demonstrate SERS as a facile approach for evaluating the integrity and crystallinity of semiconductor thin films in real time. The selection of suitable laser conditions (e.g. excitation wavelength, power density) for in-situ SERS measurement that result in minimal laser perturbation to data acquisitions will be discussed. The semiconductor thin films on SERS-active Au substrate was then served as an overlayer SERS platform for studying vibronic processes at the semiconductor surfaces. Preliminary data on the surface functionalization of the Cd-based semiconductor thin films will be presented to show that the adsorption of benzenethiol onto CdS, CdSe and CdTe can be monitored in situ in aqueous solution at ambient pressure. Potential-dependent shift of the Raman vibration modes has also been investigated to bring further insight to the surface binding mechanism at these semiconductor surfaces.

Keywords: Electrochemistry, Surface Analysis, Surface Enhanced Raman
Application Code: Materials Science
Methodology Code: Vibrational Spectroscopy
2,3-Dichloro-5,8-dimethoxy-1,4-naphthoquinone (DDNQ) has exhibited anti-tumor activity which can potentially be linked to the binding of the molecule to specific sites on the tumor cell. In this report surface enhanced Raman scattering (SERS) was utilized to probe the adsorption/orientation of DDNQ on gold nano-rods. Interpretation of the SERS data required a complete assignment of the vibrational modes of DDNQ and this was performed using BP86/6-31G (d, p), B3LYP/6-31G (d) basis sets and potential energy distribution (PED) calculations. In the SERS spectrum, the intensity of the C=O band was reduced and the frequency shifted relative to its solid Raman counterpart. A new band was observed in the SERS spectrum at 1617 cm⁻¹ and this band was attributed to C=O coordinating with the gold nano-rods. The solid Raman band at 1339 cm⁻¹ was blue shifted to 1350 cm⁻¹ in the SERS spectrum and a very intense SERS band was also observed at 1228 cm⁻¹. These bands are assigned to C-C ring modes. The observed shift in frequency and the reduced intensity of C=O band suggests that DDNQ was adsorbed onto the gold nano-rods via C=O group. The relative SERS intensities of the ring and C=O symmetric modes were used to determine the orientation of DDNQ. From these calculations it was concluded that DDNQ has a tilted orientation when adsorbed onto the gold surface.

Keywords: Materials Characterization, Materials Science, Raman, Vibrational Spectroscopy
Application Code: Materials Science
Methodology Code: Vibrational Spectroscopy
The combination of coherent anti-Stokes Raman scattering (CARS) and surface enhanced Raman scattering (SERS), surface enhanced CARS (SECARS), suggests a powerful label-free approach to understanding chemical interactions in cellular systems. We use a multiplex CARS microscope, synchronizing a picosecond pulsed beam for increased spectral resolution with a supercontinuum Stokes beam to obtain multiplex CARS spectra. The nonlinear properties of CARS and point-by-point scanning capabilities allow for three-dimensional imaging of the sample. To gain a better understanding of the surface enhancement mechanism of SECARS, we utilize gold (Au) nanorods of varying lengths to study the polarization dependence based on the localized surface plasmon resonance. Further results from the Au nanorods in different solutions provide insight into the plasmonic dependence and nonresonant background arising from the metallic nanostructures. The ultimate goal is to utilize the Au nanostructures with the best enhancement as antennae to amplify signals in cells, providing three-dimensional imaging and chemical information at the molecular level.

Keywords: Imaging, Raman, Surface Enhanced Raman, Vibrational Spectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
The nitrous oxide (N2O) has a long history of use as an analgesic and anesthetic, especially in dentistry. It supplements anesthesia, making it possible to reduce the doses of other related drugs and thereby facilitate elimination post-operation. Nitrous oxide is also used in some other applications such as food additives, and in the manufacturing of semiconductors. Analytical accuracy in purity determination is a critical parameter in such specific applications. Gas Chromatography is a conventional way of assaying the nitrous oxide in the United States Pharmacopeia (USP), while the European Pharmacopoeia (EP) replaced GC assay with an IR analyzer. In an effort of direct and accurate analysis of N2O in pure gas stream, we are evaluating infra red analyzers in the suppressed range of □98% N2O, which have not previously been evaluated. Several parameters such as accuracy, linearity, precision, robustness, drift and effect of selected interferences on the measurement will be studied to develop a validated analytical approach. The study enables identification of the best infra red analyzer with greater selectivity for N2O purity analysis.
Sol-gel derived thin films incorporating polarity gradients are prepared and investigated, for the first time, by single molecule spectroscopic methods. Gradients in polarity are established by an infusion-withdrawal dip-coating technique in which a substrate is coated by dipping it in a sol of time varying composition. Nonpolar phenyltrimethoxysilane (PTMOS) and polar tetramethoxysilane (TMOS) are used as the precursor silanes. The deposition reservoir is first filled with a PTMOS sol and a TMOS sol is then infused into the reservoir while the mixed sol is withdrawn, yielding a film in which the film composition gradually varies across the substrate surface. Sessile drop water contact angle measurements demonstrate, on macroscopic length scales, the presence of a wettability gradient (and hence, a polarity gradient). Raman microscopy confirms the formation of a PTMOS gradient on a microscopic scale. The Raman intensity for the phenyl C-H stretch at ~3085 cm\(^{-1}\) is found to gradually decrease along the gradient. Single molecule spectroscopic methods are used to probe gradient polarity on molecular length scales, using Nile Red dye as a polarity sensitive probe. Nile Red fluorescence is recorded simultaneously in bands centered around 590 nm and 640 nm. The ratio of the emission intensities in these bands depicts a spatial variation in the film polarity along the expected direction. The single molecule results also depict the distribution of nanoscale polarity properties, and how these properties change in time and space across the gradients. Polarity gradients of this type have important emerging applications in stationary-phase-gradient chemical separations.

Keywords: Fluorescence, Microscopy, Modified Silica, Raman
Application Code: Materials Science
Methodology Code: Molecular Spectroscopy
A key, but often overlooked, factor in the behavior of gas sampling and measuring systems is the effect of relative humidity (RH) on performance of the system. RH can have a significant effect on the sensitivity and stability of a sensor system. Even for sensors that are not sensitive to RH, the RH level may affect the efficiency of the sample delivery to the sensor. Systems sampling ambient air should always be calibrated with standards that simulate the ambient air.

But it is not easy to add adjustable values of RH to trace concentration gas mixtures. This paper discusses the various techniques for adding humidity to trace concentration gas standards without compromising the accuracy of the standard.
Abstract Text
In 2006, Satoshi Maekawa proposed a new imaging optics called the Dihedral Corner Reflector Array (DCRA) which is designed to make the floating image. DCRA consists of numerous micro-mirrors placed perpendicular to the surface of substrate. The micro-mirror array is implemented by the inner walls of minute square holes or the side of minute square pillar. We can choose two types of the structure. The primordial is based on two reflections by a pair of adjacent mutually perpendicular mirrors, i.e., a dihedral corner reflector. (S. Maekawa 2006.) Although the principal of operation is based on reflection by mirrors, the device is also transmissive and deflects light. Primordial of DCRA is not so complicated. However, it is too difficult to fabricate the DCRA by usual machining because of high aspect ratio micromachining. Therefore, in this manuscript, we fabricated the device by deep X-ray lithography due to synchrotron radiation. The characteristics of the fabricated DCRA are evaluated by the optical transmission and reflection measurements.
Advances in Micro Gas Chromatography (GC) - Applying Temperature Programming in a Micro GC to Achieve Fast, Accurate, and On-Site Analysis of Fixed Gases and Light Hydrocarbons

For many years, micro gas chromatography (GC) has been a proven and widely accepted technique for analyzing fixed gases and light hydrocarbons in the natural gas, petrochemical, and alternate energy industries. Due to additional application requirements and the demand for simplicity, it is now time to bring the technology to a new level. Temperature programming on capillary columns is introduced in the new Micro GC Fusion to expand application coverage and increase sample throughput compared to isothermal operation. Rapid temperature ramping also improves late eluting peak sensitivity and column cleaning to minimize contamination. Currently, Micro GC users require a PC and proprietary chromatography software, which introduce operation complication for some users. Micro GC Fusion incorporates an LED front panel display and web-based chromatography software allowing users to operate the instrument on a stand alone basis, without the concern of operating system compatibility and software licensing. This presentation will discuss the instrumentation changes introduced in the Micro GC Fusion to address application and operation needs for both seasoned and new GC users.

Keywords: Energy, Gas Chromatography, GC, Petrochemical
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Gas Chromatography
Simulated distillation GC method has been widely implemented in petrochemical and refinery industries, replacing traditional distillation methods. Retention times are directly correlated to boiling points, and sliced peak areas represent distilled sample amounts. In most cases, special SimDist software is needed to perform peak integration and distillation curve calculation and simulation, which runs independently from GC operation software. In this study, however, new SimDist software has been fully integrated into GC control software. GC operation conditions and SimDist parameters are stored in the same GC method. Method ASTM D2887 is presented to demonstrate new software functions.
New Products at Pittcon 2014

Comparison of Performance of Innovative Nano Stationary Phase (NSP) and Conventional Stationary Phase GC Capillary Columns for Environmental Applications

GC capillary columns prepared from conventional stationary phases (CSP) provide analyses which are mainly based on boiling points or volatilities of the analytes. The retention orders and selectivity are well established for CSP GC columns. The current investigation is related to the evaluation and application of Nano Stationary Phase (NSP) GC capillary columns for environmental sample analyses and the comparison of performance with CSP columns. Columns from different manufacturers using the same kind of stationary phase, for example 5%phenyl 95%methyl polysiloxane show a slightly different performance. Surface bonding and cross-linking of the NSP provides superior thermal stability compared to that of a CSP. Better selectivity of the NSP is due to a specific orientation of nano particles, resulting in a stronger interaction of analytes. The selectivity and stability of NSPs are the two most important properties differentiating them from conventional stationary phases. Due to their enhanced selectivity, it is possible to use shorter columns to achieve a faster analysis. The advantages of the selectivity and stability of NSP columns are illustrated for the analyses of USEPA Method 8270 CLP components and USEPA Method 610 priority PAH, on various kinds of NSP columns compared to CSP columns.

Keywords: Capillary GC, Chromatography, Environmental Analysis, Gas Chromatography/Mass Spectrometry
Application Code: Environmental
Methodology Code: Gas Chromatography
Thermogravimetry (TG) is a well-known technology for the investigation of thermal decomposition. The technology is used to characterize temperature dependent material properties, evaluate thermodynamical conversions and thermophysical parameters. It is used in material research, especially in polymer industry. For more advanced applications a chemical analysis of the evolved gases is required. The coupling of mass spectrometry (MS), as a fast on-line gas analyzer, with TG is a well-known analytical method. Commonly a MS with electron impact ionization is used. Currently available TG-MS systems have problems in showing the composition of the degassing organic compounds, because the conventional electron impact ionization produces intense fragmentation, making the interpretation of spectra difficult.

With a novel photo ionization source a soft ionization is possible which reduces the amount of fragments considerably. The novel and highly brilliant VUV light source, based on an electron beam pumped rare gas excimer lamp (EBEL), can be used as an alternative to other soft ionization sources. A combination of TG with the new Time of flight (TOF) mass spectrometry was shown at the last Pittcon. This poster will show measurement results of different oil samples, which are difficult to characterize with conventional methods. The advantages of the new soft ionization source are discussed as well as the advantages of using a TOF instead of a conventional quadrupole mass spectrometer.

**Keywords:** Hydrocarbons, Mass Spectrometry, Thermal Analysis, Time of Flight MS

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

**Methodology Code:** Thermal Analysis
The performance advantage of core-shell or Fused-Core® columns over U/HPLC columns packed with fully porous particles of the same or even 25-30% smaller particle size, is now widely accepted. Despite lacking a well understood theoretical basis for the improved performance, all major HPLC column manufacturers now provide core-shell columns packed with (nominally) 2.7 micron particles for the analysis of compounds of low molecular mass.

Although most compounds analyzed by HPLC have a mass below 1500 Da, there is a growing need to improve the performance of HPLC columns for the separation of biopolymers by reversed phase and other LC modes. Although at least one large pore size core-shell particle has been introduced in recent years, the analytical biochemists has few options to select from different particle and pore sizes, and generally lacks the ability to test the effect of alternative bonded phase selectivities.

In this presentation we discuss the characteristics and performance of the BIOshell™ C18 and CN column line for peptide analysis using 2.7 and 5 micron particles with 160Å pores. For protein analysis by reversed phase, the new BIOshell A400 Protein C4 columns are based on 3.4 micron particles with 400Å pores. The pore size of BIOshell A400 Protein C4 columns provides unhindered access to proteins as large as 400,000 Da, while the 3.4 micron particles size allows for fast and efficient analysis of proteins at moderate pressure in traditional HPLC systems.

We will demonstrate the performance of BIOshell columns at elevated temperatures and within the pH range of 2 - 9 for C4 columns, while other performance characteristics such as protein recovery, sample capacity, peak capacity, etc. will be highlighted as part of examples showing improved separations of proteins and peptides versus columns packed with fully porous particles.

Keywords: Bioanalytical, Biopharmaceutical, HPLC, HPLC Columns
Application Code: Bioanalytical
Methodology Code: Other (Specify)
Superficially porous particle technologies (Fused-Core®, core-shell) have rapidly gained acceptance in general high-performance liquid chromatography (HPLC) and ultra-high performance liquid chromatography (UHPLC) practice over the past several years due to improved efficiency relative to comparably-sized fully porous particles. The application of Fused-Core 2.7µm particles has exploded in popularity as an alternative to sub-2µm porous particles because UHPLC performance (>200,000 plates per meter) can be achieved at much lower backpressures. 5µm Fused-Core particles deliver almost twice the performance of common 5µm fully porous particles at the same low backpressure and equal performance to 3µm fully porous particles at much lower back pressure. HPLC columns with 5µm Fused-Core technology provide greater ruggedness and better performance (>150,000 plates per meter) at low pressure, making these columns ideal for routine HPLC in a production QC environment. Immediate performance improvement can be achieved without resorting to either smaller particle columns or higher pressure instruments.

Fused-Core columns are comparable to fully porous columns in terms of efficiency and selectivity for easy method transfer. Evidence will be shown that same selectivity is maintained for the same phase chemistry on Fused-Core silica. Columns with original Fused-Core 2.7µm technology remain ideal for research and development of methods on modern, optimized U/HPLC instruments, while columns with larger 5µm Fused-Core technology are well suited for classic HPLC instruments requiring either no or minor optimization.

**Keywords:** HPLC Columns, Liquid Chromatography, Pharmaceutical

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography
Physical Measurements

A Numerical Evaluation of Iterative Solvers for the Solution of Static Light Scattering Problems

Static light scattering, also known as laser diffraction, is a common technique for determining particle size. Determining particle size from a measured laser scattering or diffraction pattern is effectively an inverse problem, i.e.

\[ b = A x + \epsilon \]

- \( b \): scattered light pattern (known)
- \( A \): refractive index matrix (known)
- \( x \): particle distribution (unknown)
- \( \epsilon \): noise (unknown)

Due to the nature of \( A \), determining \( x \) in the equation above is known as ill-posed inverse problem. In other words, the problem does not have unique solution and there are infinite set of likely solutions for a finite set of intensity data. Thus it is necessary to apply an iterative solver (asymptotic numerical method) for the most likely solution in terms of physical rationality. In recent years a number of solvers for the ill-posed inverse problem have been developed. This paper compares the performance of two solvers, HyBR (Hybrid Bidiagonalization Regularization) and MRNSD (Modified Residual Norm Steepest Descent), for the case of analyzing particle size distribution. To evaluate the solvers, we consider that theoretical light scattering pattern from a sample with 3 peaks (picket fence) which corresponds to the case of mixing three different size polystyrene latex particles, each of which has narrow size distribution. With these numerical simulations, we discuss
- Behavior of rational residuals when varying the number of iterations
- Accuracy of obtained size peak positions and mixing ratios

Finally, we propose a combined method which includes the advantages of both methods.

Keywords: Light Scattering, Particle Size and Distribution

Application Code: Materials Science

Methodology Code: Physical Measurements
Intensifying use of nanoparticles in different products and technologies increases dramatically a risk of contamination of environment, drink water and food with potentially toxic materials. In the same time, available analytical techniques are not well suitable for detection and analysis of these materials, especially at low concentrations and natural environments.

An application of high resolution surface plasmon resonance imaging (SPRI) for detection and analysis of nanoparticles at extremely low concentrations in aqueous solutions will be discussed. Recent works demonstrated that nanoparticles adsorbed on the surface with excited surface plasmon wave scatter enough light to be detected by SPRI, thus giving a unique possibility to detect the adsorption of each single nanoparticle within a relatively large (1-2 mm^2) sensor surface in the real time. Such an extremely large surface sensitivity (just a few nanoparticles per mm^2) leads to correspondingly low detection limit in volume, provided that nanoparticles in volume eventually diffuse and adsorb to the sensor surface. That’s why both surface modification of a sensor and nanoparticle have a strong influence on the frequency of nanoparticles binding and their further behavior on the surface. This provides a possibility to apply the concept of analyte - receptor interaction for detection of single nanoparticles with SPRI-transducing. The results of ongoing investigation on the influence of different sensor surface coatings on their interaction with various practically important engineered nanoparticles will be presented.
Abstract Text
The development of protein therapeutics has created a uniquely powerful tool to address clinical requirements unmet by traditional pharmaceutics. However, instability and resulting aggregation of therapeutic proteins has the potential to yield inactive and/or immunogenic aggregates. These aggregates range from 0.1 microns to 10's of microns in size. There is a need to know the particle size distribution and concentration of these particles. Currently, instrument manufacturers are trying to utilize both old and new technologies to supply this information. The challenges they present are difficult. As stated above, the expected sizes will range over 3 or more orders of magnitude while the concentration is expected to range over many more orders of magnitude than size. We have previously described a new technique for this application, Focused Light Obscuration, which is a form of optical particle counting. This technique was shown to produce high resolution particle size distributions down to 0.15 microns in addition to particle concentrations with high statistical accuracy over a six decades. In order to extend the size range and the concentration range that can be measured, we have combined Focused Beam Light Obscuration with traditional SPOS. This new combined technique allows one to size and count particles over a size range of 0.2 to 100 microns and a concentration range of up to 8 orders of magnitude. In this study, the new method will be applied to both stable and unstable proteins and compared to other methods in terms of the statistical accuracy of the results.

Keywords: Particle Size and Distribution, Protein
Application Code: Bioanalytical
Methodology Code: Physical Measurements
## Physical Measurements

### GEI Point Determination Thanks to Microrheology

This work presents a new technique of passive microrheology for the study of the microstructure viscoelastic properties and the gel point transition for 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. Our technique is based on Diffusing Wave Spectroscopy. It consists of Dynamic Light Scattering (DLS) extended to an opaque media. In a DWS experiment (more precisely Multi Speckle-DWS in our case), a coherent laser beam is applied to the sample containing scatterers (particles, droplets, fibers...). The light is multi-scattered by these scatterers, which leads to interfering backscattering waves. An interference image is detected by a multi-pixel detector. In dynamic mode, the scatterers motion (resulting from thermal energy) induces spot movements of the speckle image. A patented algorithm enables the treatment of this speckle image in order to determine the scatterers mobility in terms of speed and displacement which are directly related to the samples viscoelastic properties. The determination of the Mean Square Displacement (MSD) curve enables to characterize completely 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 105 seconds. This work presents a rescaling data processing known in rheology as time cure superposition, to determine the gel point transition and gel strength parameters. Results will show the automatic determination of the gel point versus time for gelling systems like yoghurt or gelatin, gel point versus concentration for xanthan polymer, cross-linker concentration effect on the gel point.

### Keywords: Characterization, Instrumentation, Laser, Rheology

### Application Code: Other (Specify)

### Methodology Code: Physical Measurements
The interface between an oil and water provides a unique junction for the adsorption and assembly of charged macromolecules, polymers and coated nanoparticles. With their hydrophobic backbones that coil and bend into a multitude of conformations, and the fickle hydrophilic functional groups whose characteristics can vary widely depending upon the aqueous phase composition, the molecular nature of adsorption and assembly of macromolecules at a liquid surface is largely unknown. Even less is known about how macromolecules can coat and alter the adsorbing properties of inorganic nanoparticles at such an interface. This presentation will describe our most recent spectroscopic studies of a variety of charged polymeric materials and coated nanoparticles as they assemble at the oil-water interface.
The current environment for research, whether academic or industrial seems to be in the mode of “do more with less”. The competition for academic funding is more severe, and the goals for industrial research are cast in an ever shorter time frame. In order to continue finding satisfaction in our profession we need to actively explore alternative models for research groups. One possible answer to these increasingly severe constraints lies in research collaborations between industrial and academic investigators. My specific example involves retired industrial scientists. This group of scientists represents an opportunity which is a win-win scenario for both the academic and the retiree. A retired industrial scientist brings a different perspective to the functioning of a research group, acting as both a mentor and a partner. I will illustrate the benefits of such a collaboration with results from a polymer physics group, and two solid state materials groups, involving nanofibers, solar cell materials, and terahertz generation.

**Keywords:** Energy, FTIR, Materials Characterization, Materials Science

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

**Methodology Code:** Vibrational Spectroscopy
SHG and SFG spectroscopies have demonstrated unparalleled selectivity in studies of the structure of molecules at interfaces. While it does not provide surface or interface selectivity, multivariate analysis of matrix-formatted broadband spectroscopy data seeks similar objectives: isolation and characterization of the essential components of complex systems. They can play a unique role in the analysis of complex systems formed by self-assembly and held together by non-covalent interactions. In this presentation, a novel type of multivariate analysis of matrix-formatted Raman spectra, collected as phospholipid mixtures containing DMPC (C14) and DHPC (C7) in water form aggregates of various morphologies between 7 [degree]C and 43 [degree]C, will be presented and discussed. Mixed lipid aggregates form intriguing soft materials. For example, at temperatures near 37 [degree]C, they form three-dimensional networks that reversibly gel water. These materials are finding applications in a variety of fields from membrane protein crystallization to separations. The multivariate analysis separates several lipid spectra from those of background species such as sample preservatives and residual fluorophores, uncovering spectral features that reflect lipid interactions with water as well as the well known structural changes that indicate phase transitions of the alkyl chains.

**Keywords:** Array Detectors, Data Analysis, Lipids, Raman

**Application Code:** Other (Specify)

**Methodology Code:** Vibrational Spectroscopy
## Pittsburgh Spectroscopy Award

### Enhancing Molecular Structural Information in Nonlinear Vibrational Spectroscopy

Even-order nonlinear spectroscopies are valued for their sensitivity to interfacial structure as they are capable of discriminating molecules from adjacent bulk phases based on symmetry. Visible-infrared sum-frequency generation (SFG) spectroscopy is a second-order technique that additionally harnesses the sub-molecular structural sensitivity of a vibrational spectroscopy by tuning the infrared laser over molecular resonances. As a result, over the past two decades, SFG spectroscopy has been successfully applied to a wide variety of solid, liquid, and vapor interfaces, revealing signatures of the molecular organization. A remaining challenge is to interpret the spectral response to arrive at a quantitative understanding of the surface structure. Our group has been focused on developing experimental and modeling techniques to aid in the molecular interpretation of the SFG response. For small molecules, this includes grid computing-based searches to validate candidate orientation distributions based on the experimental data. For larger molecules with additional conformational flexibility, we employ molecular dynamics simulations to assist and further refine our efforts to interpret the SFG data. Our most recent efforts explore the use of phase-resolved SFG spectra in order to develop more sensitive functions for scoring trial molecular orientation distributions. Our goal is to develop tools that are scalable to molecules of arbitrary complexity. This talk will provide some examples to illustrate our path towards this direction.

**Keywords:** Biospectroscopy, Data Mining, Infrared and Raman, Spectroscopy

**Application Code:** Materials Science

**Methodology Code:** Vibrational Spectroscopy
Interfacial interactions begin to influence flow rates of liquids through pores when the physical dimensions of the pores reach the nanoscale. For pores less than 100 nm, one can see a significant amount of flow enhancement due to slip flow, which arises from weak interactions between the liquid and the surface. We study how the hydrophobicity of the surface affects slip flow of water through chemically modified silica colloidal crystals. The findings have implications for ultra-high performance liquid chromatography.

Keywords: Biopharmaceutical, Capillary LC, HPLC, Materials Science
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
The use of mass spectrometry in clinical analysis has grown dramatically over the last few years, in part because of the tremendous sensitivity and selectivity offered by tandem mass spectrometry in combination with rapid chromatographic separation. New and innovative mass spectrometry technologies have contributed to that growth, since they offer capabilities to solve even more challenging clinical problems as well as to perform clinical analyses more rapidly. This talk will provide a perspective on the increasing role of mass spectrometry in clinical analysis and on the potential for innovative approaches to expand that role over the next few years. Topics will include small molecule and proteomic applications, as well as innovations in sample preparation, separation, ionization, and mass analysis.

Keywords: Clinical Chemistry, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry, Metabolomics
Application Code: Clinical/Toxicology
Methodology Code: Mass Spectrometry
The identification and quantification of biologically relevant proteins in complex matrices often require the use of high performance nanoLC separations to achieve detection limits in the pM range. Typical flow rates in the 200-300 nL/minute range and 60-90 minute gradient separations afford high sensitivity, but confound the development of rapid and robust clinical proteomic assays that require increased throughput and ease of use. This presentation will describe the development of modular nanospray columns, PicoChips, and their utility for improving the throughput and reliability of quantitative proteomic assays. A novel column switching schema is demonstrated that triples the throughput and simplifies the HPLC pump configuration compared to previous designs. The performance characteristics of the modular chip based nanospray columns are evaluated for the quantitative analysis of proteins in blood, spinal fluid, and other complex matrices. Finally, we demonstrate how the modular columns create new opportunities for improving the reliability, reproducibility, and throughput of clinical assays that require nanoLC separations.
Clinical Analysis: The Next Frontier in Mass Spectrometry

Imaging Metabolites and Metabolic Pathways in Cancer

In 2011 metabolic dysfunction was added as one of the distinct hallmarks of cancer. Using ultrahigh mass resolution to ensure all metabolite ions are clearly resolved we have initiated a metabolite imaging MS investigation to establish the diagnostic and biological potential of MALDI imaging MS for analyzing endogenous metabolites in human cancer tissues.

Ultra-high mass resolution MALDI imaging MS datasets of metabolites have been recorded for thyroid cancer and triple negative breast cancer. The ultra high mass resolution and accurate mass capabilities of the FTICR mass spectrometer enabled most metabolites to be fully resolved. For example the metabolite MALDI imaging MS data obtained from triple negative breast cancer contained more than 4000 distinct peaks, S/N>5, in the m/z range 70-1000. The mass spectral data was then filtered using the human metabolite database. A peak detection algorithm was applied to the mass spectra, recording the m/z and intensity of each peak; those peaks detected within 1 ppm of the theoretical m/z of the [M-H]- metabolite ions were retained. The filtered metabolite data was then analyzed according to known metabolic pathways.

Non-negative matrix factorization (NNMF) was then applied to the datasets. NNMF is a multivariate technique used in text mining that is adept at identifying latent topics based on the co-occurrence of words. When applied to imaging MS data NNMF identifies latent molecular classes based on the co-occurrence of the detected ions. NNMF revealed multiple distinct classes, which includes viable tumor, tumor interface, stroma, and background. To provide an indication of the contribution of the different metabolic pathways the factor spectra were filtered using the human metabolome database, and which revealed molecular signatures consistent with the altered metabolism of the Warburg effect.

Keywords: Clinical/Toxicology, Imaging, Mass Spectrometry, Metabolomics

Application Code: Clinical/Toxicology

Methodology Code: Mass Spectrometry
MALDI-TOF in Clinical Microbiological Analysis

Until recently, microbial identification in clinical diagnostic laboratories has mainly relied on conventional phenotypic and gene sequencing identification techniques. The development of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) devices has revolutionized the routine identification of microorganisms in clinical microbiology laboratories by introducing an easy, rapid, high throughput, low-cost, and efficient identification technique. This technology has been adapted to the clinical diagnostic laboratories and has the potential to replace and/or complement conventional identification techniques for bacterial, and slow growing mycobacterium and fungal organisms. MALDI-TOF MS has been successfully adapted for the routine identification of microorganisms in clinical microbiology laboratories. This revolutionary technique allows for easier and faster diagnosis of human pathogens than conventional phenotypic and molecular identification methods. Integration of rapid diagnostic testing via MALDI-TOF with antimicrobial stewardship team (AST) intervention assists in early organism identification, customization of antibiotic therapy and improvement in patient outcomes. Examples will be discussed where MALDI-TOF with AST intervention decreased time for organism identification, and time to effective and optimal antibiotic therapy.

- **Keywords:** Identification, Mass Spectrometry, Medical
- **Application Code:** Clinical/Toxicology
- **Methodology Code:** Mass Spectrometry
Clinical Analysis: The Next Frontier in Mass Spectrometry

Challenges of Newborn Screening: Past, Present and Future

Newborn screening (NBS) allows identification of newborns with treatable, serious medical conditions including cystic fibrosis, endocrine disorders, immunodeficiencies, hemoglobinopathies and inborn errors of metabolism (IEMs). The goal of NBS is to identify at-risk infants and start treatment before symptoms develop. Within days of birth, blood is collected on filter paper and transported to a distant laboratory for analysis. The small specimen size, large number of specimens, the need for rapid turn-around and stability of the analyte during transport under wide-ranging conditions are challenges for any testing method.

In the past, newborn screening testing was restricted to those disorders for which a sensitive, high throughput test was available; each test identified a single disorder. Currently, MS/MS is used to quantify amino acids and acylcarnitines with a single test screening for a number of disorders of amino acid, organic acid and fatty acid metabolism. Despite the increased number of disorders identified by MS/MS, challenges remain, among them, the lack of testing for some treatable IEMs. New treatments have been developed for lysosomal storage diseases, making these disorders candidates for newborn screening. It is likely that the addition of lysosomal enzyme assays to newborn screening will bring new challenges.

Keywords: Clinical/Toxicology, Tandem Mass Spec
Application Code: Clinical/Toxicology
Methodology Code: Mass Spectrometry
Two different techniques have been developed for different PEDs. One is the chiral analysis of stimulants. The other is an untrasensitive technique (parts per trillion to sub ppt) to detect steroid metabolites and other drugs of abuse in urine called PIESI (paired ion electrospray ionization) MS.

Keywords: Biological Samples, Chiral, Drugs, GC
Application Code: Other (Specify)
Methodology Code: Liquid Chromatography/Mass Spectrometry
The consumption of certain compounds used for performance enhancement can be considered as drug abuse and are banned in sport competitions. Every year, new compounds and their metabolites are added to the list of prohibited substances, requiring strict control of their consumption by athletes. The diversity of their physicochemical properties, the necessity for hydrolysis of conjugated forms of analytes, their pharmacology and the required minimum performance limits for determination of those compounds all contribute to make their analysis challenging. Solid phase microextraction (SPME) is a well-established sample preparation technique. The extraction mechanism is either based on the distribution coefficient, when equilibrium between the extraction phase and the sample matrix is established, or on a mass transfer rate at pre-equilibrium. SPME is suitable for automation, it requires low consumption of organic solvents, and is a simple, relatively less expensive method, considering the reusability of the sampling devices. In this presentation the role of SPME in doping analysis as an alternative sample preparation method will be discussed by showing various SPME applications for analysis of the banned compounds. Firstly, a fully automated, high-throughput method based on thin-film solid phase microextraction and liquid chromatography mass spectrometry which was developed for simultaneous quantitative analysis of 110 doping compounds in urine, selected from ten classes and varying in physical and chemical properties will be presented. As a second study, use of commercial SPME fiber for direct immersion microextraction of steroids from serum samples followed by headspace on fiber derivatization and GC-TOF determination will be presented. Thirdly, determination of doping compounds in urine samples by thin-film solid-phase microextraction and direct analysis in real time (DART) coupled with tandem mass spectrometry will be shown and discussed.

**Keywords:** Bioanalytical, High Throughput Chemical Analysis, Mass Spectrometry, SPME

**Application Code:** Bioanalytical

**Methodology Code:** Sampling and Sample Preparation
Doping to gain an unfair advantage in sport has been practiced since the time of the ancient Olympics. As the performance-enhancing and potentially harmful effects of doping agents became well known, several sporting federations instituted bans on these agents followed by implementation of testing to detect their abuse. This resulted in athletes resorting to newer doping agents (eg: development of designer steroids), abusing pharmaceutical agents for which detection methods had not yet been developed (eg: erythropoietin {EPO}) or using sophisticated procedures designed to evade current testing procedures (eg; microdosing and masking agents). This talk will provide a brief overview on the development of the above doping practices and the testing procedures and instrumentation used to detect them. Among the methods presented will be isotope ratio mass spectrometry (to detect the use of exogenous testosterone) and isoelectric focusing-polyacrylamide gel electrophoresis (IEF-PAGE -to detect the use of EPO). Indirect detection methods involving longitudinal profiling such as the ‘Athlete Biological Passport’ will also be discussed. In order to illustrate the tangible influence of doping agents and reliable testing methods on sporting records, case studies from the existing literature will be presented. Finally, the benefits of intelligence gathering and collaborative efforts between anti-doping and law enforcement agencies will be discussed.

Keywords: Bioanalytical, Isotope Ratio MS, Mass Spectrometry, Toxicology
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
There is a growing interest in the use of saliva as a bioanalytical tool for pharmacokinetics, drug monitoring, or toxicological studies. Biological fluids traditionally used for such studies include blood, plasma, and urine. While urine provides time-weighted average concentrations of the analytes, blood and plasma permit an estimation of the circulating level of the analytes. Saliva consists of 99.5% water with electrolytes, mucus, proteins, and small molecular compounds, which may be transferred by diffusion from the blood. Compared with blood, the collection of saliva is safe, simple, noninvasive, and does not require any trained personnel. Detection of analytes of interest in saliva is challenging because they are present at very low concentrations since only the free form of analytes can be found. Solid-Phase Microextraction (SPME) is a fast, sensitive, and solvent-free sample preparation technique, in which the amount extracted is proportional to the free fraction of the analyte. We have recently demonstrated that it is possible to extract hundreds of analytes with a wide range of polarity using a biocompatible SPME fiber from human saliva[1]. During the presentation we will discuss new directions in vivo SPME sampling of saliva as an approach to provide reliable and accurate determination of doping substances.

References
1. Bessonneau V., Bojko B., Pawliszyn J. Bioanalysis 2013, 5, 783-792

Keywords: Bioanalytical, Liquid Chromatography/Mass Spectroscopy, Sample Preparation, SPME
Application Code: Bioanalytical
Methodology Code: Sampling and Sample Preparation
Implementation of Quality by Design in development allows for improved understanding of the analytical method focusing on robustness and ruggedness designed with end user in mind thereby facilitating methods transfer and provides opportunities for continual improvement. This allows for reduced chance of method failures during release/stability testing, aids OOS investigations, and ultimately increases quality and reduces costs. By integration of elements of Analytical Quality by Design, into the development strategy the critical sources of analytical variability (continuous and discontinuous) are identified thru risk assessments, measured and understood so that they can be controlled with the appropriate control strategy. Quality by Design implementation is aligned with a risk and science based approach to methods development and methods validation. The outcome of the risk analysis allows for the identification of the potential critical analytical method variables that could be evaluated using a statistical design of experiments. Design of experiments (DOE) is used to provide the most efficient and statistically sound approach to evaluate multiple method variables and their responses (critical method attributes). This provides an excellent opportunity for determination of optimum conditions generated from a limited number of experiments used in the DOE study. Also, statistical design facilitates an in-depth understanding of the method and justifies the choice of ranges for method variables and finds a robust (optimum) region for the final method. Finally an analytical method design space could be developed encompassing the operable ranges for the potential critical analytical method variables and their interactions and suitable control strategies can be implemented.

Keywords: Optimization, Pharmaceutical, Quality, Statistical Data Analysis
Application Code: Pharmaceutical
Methodology Code: Separation Sciences
Liquid Chromatography is undoubtedly the most commonly used technique for small molecule analysis. Chromatographic method development involves understanding of intrinsic molecular properties, knowledge of separation science along with related instrumentation. After the chromatographic method development, the method must be validated and transferred for its potential commercial operation use. The elements of validation, transfer along with the current thoughts on lifecycle management will be discussed in this presentation. Relevant quality guidelines along with approaches for stability indicating properties and method remediation will be presented in details. Strategies and case studies of selected impurity analysis will also be highlighted.

**Keywords:** Analysis, HPLC, Liquid Chromatography, Liquid Chromatography/Mass Spectroscopy

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography
Biomolecules exhibit very low diffusivities and are ideally suited to benefit from UHPLC. However, uptake of the technology for biological separations has been restrained until now by a limited selection of column choices. This talk will discuss development of UHPLC methods as a replacement for classical HPLC methods in the analysis of biomolecules. The variety of HPLC methods used in the control and analysis of therapeutic proteins will be discussed, as will an assessment of the availability of UHPLC methods to replace the legacy HPLC methods. Data from several methods will be presented to highlight the potential upside to UHPLC for the development of therapeutic proteins. Challenges associated with the implementation of UHPLC methods and new approaches for the development of those UHPLC methods to ensure multiple instrument applicability will be outlined.

**Keywords:** Biopharmaceutical, HPLC, Validation

**Application Code:** Bioanalytical

**Methodology Code:** Liquid Chromatography
Analytical Strategies in Biosimilar Development

Biosimilar development involves an iterative target directed approach leading to a manufacturing process that delivers a highly similar product. Subsequently, similarity to the originator product is demonstrated by a comprehensive comparability program. A key element of this comparison is extensive physicochemical and biological characterization, using an array of state-of-the-art analytical techniques. On the basis of this characterization, a tailored pre-clinical and clinical program is designed to demonstrate and confirm biosimilarity.

In this talk an analytical strategy for the development of GP2013, a Rituximab biosimilar candidate will be discussed. Examples from analytical cell line and process development support as well as comparability data to the Originator drug will be shown.

Keywords: Characterization, HPLC, Mass Spectrometry
Application Code: Other (Specify)
Methodology Code: Other (Specify)
The success of biologics in both medical field and financial market has led the pharmaceutical industry to ramp up investment in biologics R&D. In the meantime, the off-patent scenario for several blockbuster biologics in the next five years has stimulated the effort in establishing capabilities for developing and commercializing biosimilars. In either case, comprehensive characterization is one of the most critical pieces of biologics analytics in the development of innovative drugs or biosimilars. Due to the complex nature of biologics which oftentimes consist of millions or billions of isoforms, characterization of molecular isoforms is an ever challenging task that is heavily dependent on technical advances in the related areas. This presentation will review the major isoforms in protein therapeutics especially in monoclonal antibodies. Common practice of molecular isoform characterization in pharmaceutical industry will be described too. In addition, recent trends in molecular isoform characterization using electrophoresis, liquid chromatography, and mass spectrometry will be presented.

**Keywords:** Bioanalytical, Biopharmaceutical, Electrophoresis, Mass Spectrometry

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
The recent advancement in molecular engineering has greatly facilitated the design and development of DNA logic circuits for a variety of novel applications in molecular medicine. For example, cell types, both healthy and diseased, can be classified by the inventories of proteins present and absent on their surfaces. Here, we report the design and implementation of programmable aptamer-based logic circuits that can assess an inventory for individual cells and respond based on more than three parameters. These programmable nanostructures combine aptamers that detect specific surface proteins with "toehold" DNA catalysis to create a biocircuit that, via a series of AND, OR and NOT logic decisions, creates an output that depends on what proteins are present and absent on that individual cell. We will report a prototype of such a biocircuit that profiles four coexisting cell surface markers, several found on cancer cells, with the potential of a diagnostic signal and/or targeted photodynamic therapy as the outcome. In addition, we take the molecular DNA circuit further to mimic biological functions such as acquired immune system (AIS) which is able to mount a significant and long-lasting defense based on three key functions: recognition, response and memory. Focusing on the AIS, we have reproduced such functions using DNA building block. More specifically, we have developed a biomimetic system in which DNA and enzymes are used as artificial analogs that systematically and macroscopically mimic real cellular activities triggered when a pathogen successfully evades the innate immune defense system. Our approach provides a rational and simplified bottom-up strategy for the biomimetic design of a complex biological network.

Keywords: Bioanalytical, Biosensors, Biotechnology
Application Code: Bioanalytical
Methodology Code: Sensors
We developed an evolutionary engineering method to generate DNA aptamers that bind to target proteins with extremely high affinity and specificity by a genetic alphabet expansion system. Genetic information flow in the central dogma relies on only the four nucleobase components, ruled by A-T(U) and G-C pair formations, which in turn constrain the Darwinian evolution of nucleic acids as functional molecules. Thus, the expansion of the genetic alphabet by introducing an artificial extra base pair (unnatural base pair) into DNA could provide a new biotechnology for generating nucleic acids and proteins with increased functionality. Recently, we developed an unnatural base pair between hydrophobic 7-(2-thienyl)imidazo[4,5-b]pyridine (denoted by Ds) and 2-nitro-4-propynylpyrrole (denoted by Px) that exhibits high selectivity and efficiency in PCR.

We applied the Ds-Px pair PCR system to DNA aptamer selection by developing a new evolutionary engineering method (SELEX or in vitro selection). In the method, we prepared a DNA library containing the hydrophobic Ds bases as a fifth base in its random sequence region, and the DNA library was amplified by PCR involving the Ds-Px pair system. We demonstrated DNA aptamer selection targeting human vascular endothelial cell growth factor-165 (VEGF-165) and interferon-α and obtained DNA aptamers that bind with Kd values of 0.65 pM and 38 pM, respectively, which are >100-fold improved over aptamers containing only natural bases. Our data showed that the increased complexity of genetic information, with only a few of the fifth hydrophobic bases, could augment nucleic acid functionality through evolution, thus providing a powerful tool for creating new functional nucleic acids toward diagnostic and therapeutic applications.
Engineered Antibody-Mimics with Increased Affinity and Selectivity

Peptide-Based Biological Recognition Elements for Sensing Applications

Biomolecules offer exquisite discrimination in their interactions with proteins, peptides and small molecules, providing considerable potential in sensing applications. Central to sensing is the selective recognition of biological or chemical species of interest. Peptides and nucleic acids are been explored in addition to conventional antibodies and nanobodies as recognition elements. The recognition elements are interfaced with transducers to provide an output. Nanomaterials such carbon nanotubes, graphene or metallic nanoparticles exhibit unique electrical and optical properties and serve as excellent transducers. These recognition elements can be functionalized onto these nanostructured transducers for the rapid detection of target analyte. We have explored the use of peptides recognition elements for sensing biological or chemical targets. In my talk, I will highlight approaches to selecting peptide-based recognition elements, functionalizing nanomaterials for creating biosensors, and demonstrating selective detection of biological or chemical targets. Comparison of the peptide-based sensing approach to antibody-based methods will also be demonstrated.

Bioanalytical, Biosensors, Nanotechnology, Peptides

General Interest

Sensors
Engineered Antibody-Mimics with Increased Affinity and Selectivity

Epitope Targeted Synthetic Protein Capture Agents

The approach of epitope targeted peptide-based protein capture agents, based upon sequential in situ click chemistry, will be described. The basic approach is to develop protein capture agents that exhibit monoclonal antibody like selectivity and affinity, but are exact chemical structures with built in stability against the physical conditions or biomolecular challenges (proteases) that can damage antibodies. The concept uses a chemically synthesized fragment of the target protein (the epitope) as a highly selective catalyst for promoting the covalent coupling of an azide-presenting peptide with an acetylene-presenting peptide to form a triazole linkage. Only those peptides that fit onto the protein surface in just the right way are so catalyzed. Capture agents against approximately 25 targets have been developed, including antigens used for HIV and malaria diagnostics, as well as various oncology targets. The use of these capture agents for both in vitro diagnostics, as well as for altering protein function, will be discussed.

Abstract Text

Keywords: Combinatorial Chemistry, Immunoassay, Proteomics
Application Code: Genomics, Proteomics and Other 'Omsics
Methodology Code: Chemical Methods
Engineered Antibody-Mimics with Increased Affinity and Selectivity
Selective and Reversible Biodetection in Complex Matrices – Synergistic Roles of Biology and Electronics

For over a century, antibodies have been attracting the attention of biologists and chemists due to their biological binding functionality with highly tailored selectivity. Until recently, engineered bioreceptors have been outperforming antibodies only in their stability and ease of production but not in their selectivity and reversibility of response. In our invited talk, we will discuss results of our recent progress on the development of biosensors with new capabilities based on aptamer bioreceptors. One of our studies was focused on the enhancement of the biodetection selectivity. We performed detection of several model analytes of homeland security and public safety interest in the presence of up to 1000 fold overloading with interference species. The knowledge from these experiments provides important insights for the rational design of the future biosensors with significantly reduced or eliminated interference effects. Another study was focused on the improvement of the reversibility of the biosensors. We achieved 365 bind/release cycles of reversible operation of immobilized aptamers and demonstrated the high stability of the binding constant over these measurements. Scenarios where the multiple biodetection cycles over the long periods of time of months and years will be of critical importance can include unattended operation of biosensors in remote areas or as sensing nodes of a distributed sensor network.

Abstract Text

Keywords: Bioanalytical, Biosensors, Sensors
Application Code: Bioanalytical
Methodology Code: Sensors
Contamination of food products with pesticides is a growing concern because of recognized adverse health effects, increasing world-wide usage of pesticides, and increasing imports of raw foodstuffs from foreign sources. Gas chromatography mass spectrometry (GC/MS) has been used extensively to quantify trace level pesticides in food matrices; the most significant challenges have been matrix interference and achievement of meaningful health-based detection limits for the compounds of interest. The QuEChERS (Quick Easy Cheap Effective Rugged and Safe) sample preparation method has helped to overcome some of the problems of matrix interference, and commercialization of QuEChERS kits has promoted widespread screening of foodstuffs for trace pesticides. Triple quadrupole GC/MS/MS operating in the Multiple Reaction Monitoring (MRM) mode has emerged as the technique of choice for analysis of trace level contaminants in complex matrices.

This presentation will present instrument configuration, operating parameters, and analytical results for analysis of trace levels of pesticides of various chemical classes in a QuEChERS extract of using the MRM mode on a triple quadrupole GC/MS/MS. Results were evaluated for calibration linearity, analytical precision, and accuracy. The effect of MS resolution on compound selectivity in a complex food matrix, and Scan/MRM analysis mode, are also discussed.
Introduction of the Latest Application of SEM/TEM to Material Sciences for Safety and Security

To design some functional devices for life security and safety, their constituent materials are controlled with nano- or sub-micro-leveled accuracy. Scanning electron microscope (SEM) and transmission electron microscope (TEM) are powerful tools for the morphological and analytical study of materials. Recently, air-sensitive materials such as a cathode of lithium ion batteries (LIB) must be manipulated under inert gas atmospheres. The latest electron microscopes are equipped with special devices to keep out air exposure. Air-sensitive materials can be successfully transported from a glove box with inert gas atmospheres to the specimen chamber of EM, and then the sample is analyzed without deforming by air [1]. In addition, techniques of sample preparation are also important for EM analysis and we have applied some cross-sectioning methods for the analysis of inner structure using SEM. To expose inner structure of samples, BIB (broad ion beam) and FIB (focused ion beam) instruments are recently utilized, in which flat cross-section and thin-section of composite materials successfully prepared without applying mechanical stress. Especially, the BIB is useful to prepare wider cross-sections with mm-order. We also have developed the special devices to keep out air exposure for the BIB [2] and the FIB [3] systems. Air-sensitive materials can be cross-sectioned and transported by compatible holders between BIB/FIB systems and Hitachi EMs. Figure 1 shows the BIB/FIB systems available to control atmosphere. In the glove box (Fig.1a), sample can be prepared and fixed onto the air protection holder (Fig.1b-c). After affixing an o-ring cap of this holder, sample can be transported by this holder to BIB/FIB systems (Fig.1d-e). The cross-sectioned sample by BIB can be transported by using same holder to SEM (Fig.1f). The thin sectioned sample created by using the FIB micro-sampling technique [4] can be transported by using the cryo-air protection FIB-STEM holder (Fig.1g) to STEM (Fig.1h). We could describe the fine structure of the cross section of LIB by EMs. In this session, we will introduce the useful function of EMs and some applications of these systems.

References

Keywords: Environmental, Fuels\Energy\Petrochemical, Microscopy, Sample Preparation
Application Code: Materials Science
Methodology Code: Microscopy
Raman analysis has been recognized to have potential for solving a variety of problems in forensic science. Examples of forensic applications including identification of illicit drugs in their containers, counterfeit currency, fibers and glitter will be discussed. Micro-XRF technology is a relatively recent introduction to the field of forensic science, trace analysis, border security, art conservation, and archeology. XRF analysis gives a rapid, non-destructive reading of the elemental composition. Several examples using micro-XRF for trace analysis, gun shot residue, glass and alloy analysis will be shown.
Nano Particle (NP) is increasingly produced for used in a wide range of industrial and consumer products. On the other hand the potential risk to be exposed to the nano-materials is globally attracted. It is important for the characterization of nano-materials to control the risk. In this lecture, we’ll make a brief introduction of X-ray analytical technologies and its application to the nanomaterial characterizations, especially from the structural information point of view.

X-ray analytical technology is one of the powerful non-destructive method to explore the materials. In particular, X-ray diffraction/scattering methods are suitable for nano-scale structural characterization including the particulate systems such as NPs. Small-angle X-ray scattering (SAXS) method enables to characterize not only the size distribution and the shape of NPs but also the information on interactions between the NPs. SAXS method is being a probe for an appropriate control of the nano-materials. Rigaku Corp. will contribute to develop the science and technology through the leading with innovation.

Keywords: Materials Characterization, Particle Size and Distribution, Quantitative, X-ray Diffraction
Application Code: Nanotechnology
Methodology Code: X-ray Techniques
Biochip Device Technology for Safety and Security

Contents of presentation: For many years, we have been challenging to develop the biosensor chip device based on microfabrication technology. From our experience, we are aiming to construct compact and mobile biosensor system which can rapidly detect and identify any biological and chemical agents at time of emergency. This system can take on a role as a safety and security monitoring system for the society and human.

Points of interest: Our system is a compact and portable system composed of multiple function units which are air sampling, rapid microfluidic PCR device for bacterium gene detection, LSPR-glyco chip for toxic protein detection, and electrochemical chip for chemical agent detection, where all the components are gathered in a box of attache case size. Gene detection and toxic protein detection are measured by PCR and LSPR, respectively, and are both measured within 15 min. Chemical agent can be successfully detected within 5 min on one of the function units.

Keywords: Biosensors
Application Code: Homeland Security/Forensics
Methodology Code: Sensors
The imaging of tissues (both in vitro and in vivo), using luminescent porous silicon nanoparticles and hybrid porous silicon nanoparticles containing superparamagnetic iron oxide nanoparticles, will be presented. Imaging of fluorescent probes in vivo is often complicated by signals coming from tissue autofluorescence. Luminescent porous silicon nanoparticle probes display unusually long-lived excited states, which allows suppression of these interfering signals by time-gated fluorescence imaging. The emission lifetime of photoluminescent porous silicon nanoparticles is in the microseconds regime (5-13 µs), whereas emission signals from organic chromophores or tissue autofluorescence is much shorter-lived (< 10 ns). A mouse injected (either IV or subcutaneously) with luminescent porous Si nanoparticles displays luminescence from both the autofluorescent tissues and the nanoparticles when imaged using a conventional steady-state fluorescence imaging system. When the same animal is imaged in a time-gated mode (acquisition of the fluorescence image 18 ns after the excitation pulse), the nanoparticles are readily observed, while minimal signals from the fast-decaying Cy3.5 imaging dye or from tissue autofluorescence are seen. Improvement of signal to background contrast ratio by > 50-fold in vitro and by > 20-fold in vivo when imaging porous silicon nanoparticles is demonstrated. The incorporation of superparamagnetic nanoparticles into the porous Si-based nanostructures allows for multimodal (fluorescence and magnetic resonance) imaging. The potential for multiplexing of images using separate porous silicon nanoparticles engineered with different properties will be discussed.

Keywords: Biomedical, Fluorescence, Imaging
Application Code: Biomedical
Methodology Code: Fluorescence/Luminescence
As a result of their high surface area and their ability to strongly absorb and emit light in the near-infrared biological transparency window, single-walled carbon nanotubes (SWCNTs) represent an ideal carrier for theranostic materials. The primary hindrance to employing SWCNTs for biomedical applications, however, entails potential toxicity issues. Through collaborative research, we have demonstrated that there exists enzymatic mechanism to biodegrade SWCNTs. This discovery may be applicable to SWCNT drug delivery platforms, where peroxidase-driven degradation may trigger the release of therapeutic cargo or breakdown the SWCNT carrier post-release. Also, employing the enzyme, horseradish peroxidase, we have demonstrated that photoluminescence (PL) is returned the short cut SWCNTs that have undergone acid oxidation; significantly, these PL-active short nanotubes may find applications in cellular imaging studies. Finally, employing chemical vapor deposition, we have synthesized nitrogen-doped carbon nanotube cups (NCNCs), which appear as stacked cup-like fibers. Employing separation techniques, shortened stacks or individual “cups” were isolated, and recently, we demonstrated that these “cups” could be selectively “corked” via the growth of gold nanoparticles. By incubating a desired “cargo” with NCNCs prior to “corking,” this nanomaterial may be able to encapsulate the material within their hollow interior cavities, and after “corking,” the “cups” could be further functionalized employing the nitrogen moieties on their basal plane. As a result, “corked” NCNCs represent an attractive nanomaterial for drug delivery and/or nanomedicinal imaging applications.

Keywords: Fluorescence, Materials Science, Nanotechnology, Toxicology
Application Code: Nanotechnology
Methodology Code: Fluorescence/Luminescence
We have two main research thrusts in the field of colloidal nanomaterials and their applications. One is based on Ln3+ doped NaLnF4 nanoparticles for optical and magnetic resonance imaging. To this purpose, we have developed synthesis techniques to make core-shell architectures which allows the optical and magnetic properties to be optimized separately. Additionally, we do surface modifications to impart compatibility with biological media and to impart binding specificity (e.g. through antibodies).

Similarly, we have developed synthesis routes for high quality PbS(e) based quantum dots with emissions around 800 nm for optical bio-imaging. Also here, core-shell architectures have clear advantages in terms of stability, quantum yield of the photoluminescence, etc.

The synthesis and basic characterisation of the nanoparticles and quantum dots will only briefly be discussed, with some emphasis on the challenges to prove the actual formation of core-shell structures. The main focus of the talk is on their use as optical and magnetic probes for bioimaging.

Keywords: Magnetic Resonance, Materials Characterization, Nanotechnology, Near Infrared
Application Code: Nanotechnology
Methodology Code: Physical Measurements
The development of novel nanoscale drug carriers requires precise measurement of the kinetics of drug release. Nonetheless, existing methods are either straightforward but inaccurate (steady-state fluorescence of Nile red) or accurate but laborious, time-intensive, and inappropriate for measuring rapid release (chromatographic or spectroscopic analysis). In order to address this gap, we have recently introduced three real-time, in situ methods to measure the kinetics of cargo release: by infrared (IR) spectroscopy, magnetic resonance, and fluorescence lifetime. The first, fiber-optic attenuated total reflection IR spectroscopy, allows real-time, direct measurement of the kinetics of both protein release and hydrogel or nanoparticle degradation (McFearin et al., Anal Chem 2011). IR spectroscopy also allows for characterization of the integrity of released protein by probing secondary structure and would be compatible with in vivo application. The second method involves encapsulating superparamagnetic iron oxide nanoparticles (SPIONs), and measuring the spin–spin relaxation time (T2) of the solution, which lengthens as released SPIONs (which shorten T2) settle out of solution (Chan et al., Anal Chem 2012). By comparing the release kinetics from nanoparticles composed of closely related particles, we have demonstrated that this method has the highest resolution of any yet reported. Finally, measuring fluorescence lifetime, which reports the proportion of released dye in both the polymer-confined and solution-dissolved populations, better distinguishes subtle differences in release profiles than steady-state measurements (Viger et al., J Controlled Release 2013). Given its applicability to hydrophobic and hydrophilic cargo, fluorescence lifetime could be employed to model release of any drug-carrier combination.

**Keywords:** Fluorescence, FTIR, Magnetic Resonance, Nanotechnology

**Application Code:** Nanotechnology

**Methodology Code:** Other (Specify)
New Directions in Water Characterization and Monitoring

Analytical and Toxicological Characterization of Emerging Disinfection Byproducts in Drinking Water

Water disinfection prevents diseases and saves lives, supported by the evidence accumulated over a century. Unfortunately, disinfection produces unwanted byproducts that may cause adverse health effects. Recent advancement in analytical and bioanalytical technologies has significantly impacted the research of disinfection byproducts (DBPs) and health effects. Health risk assessment can now be based on the reliable exposure data because analytical techniques can provide highly sensitive and specific determination of concentrations of specific contaminants, as well as based on toxicological evidence because of new bioanalytical tools. We will present our recent analytical development and application to drinking water safety research through characterization of chemical and biological (pathogens) contaminants in drinking water and toxicity of DBPs.

Keywords: Environmental/Water, Liquid Chromatography/Mass Spectroscopy, Ultratrace Analysis
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
Drinking water is a complex mixture of many thousands of naturally occurring and anthropogenic chemicals. Gas and liquid chromatography are two most popular separation techniques which are usually coupled with mass spectrometry to investigate a large group of currently known hazardous pollutants and new classes of emerging water contaminants. However, there are fundamental limitations of these hyphenated methods associated with significant resources (time and effort) required during sample preparation (pre-concentration and fractionation), low screening capacity for target pollutants and problems with the chemical identification of many currently unknown non-target water contaminants. The major gap in our knowledge about chemical contamination of water is related to highly polar components of naturally occurring organic matter or its unique products from disinfection treatments or microbial degradation.

We will present a new analytical strategy which can address tangible needs in water analysis. Our approach combines technologies of nanospray ionization, differential ion mobility separation (FAIMS) and mass spectrometry. This new technique can detect thousands of water pollutants down to a part-per-trillion concentration level in a quick and convenient fashion without pre-concentration, fractionation, chemical derivation, or column separation. Most importantly, the method provides unique capabilities for the detection and structural identification of new classes of water contaminants.

Using these unique capabilities, we were able to discover and investigate unique contaminants in groundwater and tap water in Ontario as well as bottled drinking water from major suppliers. Our research provides comprehensive information on formation, occurrence, and fate of novel drinking water contaminants which could not be previously studied by conventional techniques in water analysis.

**Keywords:** Contamination, Mass Spectrometry, Separation Sciences, Water

**Application Code:** Environmental

**Methodology Code:** Mass Spectrometry
In this study, 16 PAHs and 8 OCPs were selected as monitoring objects. SPME fiber-rotated devices were used for on-site sampling of 16 PAHs and 8 OCPs at the eight entrances of Pearl River in wet season (Aug. 13-14, 2011) and dry season (Dec. 6, 2011). Portable drills were used to rotate the fibers for on-site sampling and gas chromatography-mass spectrometry (GC-MS) was used for analysis. The concentrations of PAHs and OCPs were quantitated based on the pre-determined sampling rates. The method detection limits for PAHs and OCPs were in the range of 0.1~2 ng/L.

Results showed the free concentrations of PAHs in the surface water ranged from 92.8~198.5 ng/L (wet season), 40.9~209.9 ng/L (dry season). The free concentrations of OCPs in the surface water ranged from 14.8~87.0 ng/L (wet season) and, 39.2~101.1 ng/L (dry season). The free concentrations of PAHs in wet season ranged from 92.8~685.0 ng/L (surface water) and 84.3~419.0 ng/L (bottom water) during the flowing tide. The simultaneously determined free concentrations of OCPs ranged from 14.8~90.6 ng/L (surface water) and 19.4~87.8 ng/L (bottom water). During the ebb tide, the free concentrations of PAHs ranged from 146.9~599.4 ng/L (surface water) and 123.9~229.2 ng/L (bottom water), while the free concentrations of OCPs ranged from 33.7~104.4 ng/L (surface water) and 33.4~85.9 ng/L (bottom water). The shift of the concentrations of PAHs and OCPs at eight entrances might cause by the joint effect of runoff and tide.

Compare with traditional method, on-site sampling method is simple and rapid. It also avoids the contamination of sample, the lost of analytes during sample transportation, as well as the usage of a large amount of solvent and time-consuming sample preparation process. The on-site water sampling and monitoring method established in this study addresses the development tendency of modern analytical chemistry. It will be a promising on-site water sampling technique in the future.

Keywords: Environmental/Water, GC-MS, PAH, SPME
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Despite of the fact that solid-phase microextraction (SPME) is a well-established and advantageous sample preparation technique and in spite of all methods published during the last twenty years, SPME has not been widely implemented as a standard routine technique in the private sector and many companies and regulations rely on classic sample preparation techniques such as liquid-liquid extraction for water analysis. A number of reasons are behind this, including lack of suitable calibration methods and dissimilarities in the response of commercial fibers. In this communication, recent developments in SPME geometries, coatings, calibration strategies and applications of SPME in drinking water and wastewater samples will be reviewed. On top of that, some new strategies for implementation of SPME as routine sample preparation technique for water analysis will be presented. These strategies allow to overcome the traditional limitations to implement SPME methods in the water analysis industry.

**Keywords:** Environmental/Waste/Sludge, Environmental/Water, SPME, Water  
**Application Code:** Environmental  
**Methodology Code:** Sampling and Sample Preparation
Arsenic is one of the most important environmental agents in causing chronic human disease. Elevated levels of arsenic in drinking water affect >100 million people around the world. Chronic exposure to high concentrations of arsenic in drinking water has been associated with a wide variety of adverse health effects, most seriously, cancers of bladder, lung, urinary tract, and skin. We aim at characterizing arsenic speciation, determining the extent of human exposure to arsenic, evaluating the various treatment options for removing arsenic, and studying arsenic metabolism and health effect.
Surface enhanced resonance Raman scattering (SERRS) 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. Many methods exist and fluorescence spectroscopy dominates the detection technologies employed with different assay formats. Another advantage of SERRS over existing detection techniques is that of the ability to multiplex which is limited when using techniques such as fluorescence. We have clearly demonstrated the ability to identify the presence of a mixture of 6 analytes in solution using data analysis techniques. Here we demonstrate the development of new molecular diagnostic assays based upon SERRS which have been used successfully for the detection of bacterial infections using modified SERRS active probes. The probes have been designed to give a specific SERRS response resulting in discernable differences in the SERRS which can be correlated to a specific DNA hybridisation event.

Keywords: Bioanalytical, Nanotechnology, Surface Enhanced Raman
Application Code: Bioanalytical
Methodology Code: Molecular Spectroscopy
SERS is very attractive as a detection technology since it combines sensitivity and selectivity with the simplicity of Raman spectroscopy and is effective in aqueous suspensions and at aqueous/solid interfaces. There has been significant progress in turning this potential into practical applications with the theory and practice much better understood. The enhancement is now generally accepted to be a single process due to an analyte / plasmon interaction but the process requires chemi or physi adsorption of the analyte onto a structured surface and new analyte /metal species can form and assist charge transfer. However, SERS involves the adsorption of a molecule on a surface site on a structured metal surface often in water and in some cases on a corroded metal surface and at a molecular level these site are difficult to characterise. Ways of dealing with this are discussed. Significant progress has been made in producing reliable and reproducible substrates but they require the analyte to adsorb in a controlled manner and requiring understanding of the adsorption process including metal/analyte bonding and surface layer structure. The high sensitivity and good in situ analyte identification capability of surface enhanced resonance Raman scattering (SERRS) has made SERRS attractive for analysis of DNA and proteins with very good selectivity and detection limits which can be superior to fluorescence. The wavelength dependence of SERRS is not the same as that for SERS and additional resonance enhanced fluorescence maybe detected giving a better insight into the surface adsorption process.
The detection of illicit substances and prescribed medicines is of paramount importance for detecting substance abuse and for assessing drug dosing and therapy. The quantification of drug metabolites is useful as the drug itself may have been cleared from the system yet its metabolites remain and can be assayed to assess long-term abuse. Raman spectroscopy offers unique specificity for molecular characterization and this usually weak signal can be significantly enhanced using surface enhanced Raman scattering (SERS). Using judicious design of experiments we have recently demonstrated excellent detection and quantification for a range of drugs and biomarkers using SERS [Levene et al. (2012) Analytical Chemistry 84, 7899-7905; Mabbott et al. (2013) Analytical Chemistry 85, 923-931; Cowcher et al. (2013) Analytical Chemistry 85, 3297-3302]. In this presentation we shall report the development of SERS with machine learning methods for multiplexed quantification of drugs and their metabolites.
Early diagnosis of tuberculosis (TB), dengue fever (DF), and many other infectious diseases is essential for patient care and global infection control. Advances in diagnostics for many of these diseases are, in fact, global health priorities. In many instances, early diagnosis is synonymous with the selective detection of circulating antigens at extremely low levels in body fluids like serum and urine. This presentation will describe efforts along these lines that combine nanoparticle labels, monoclonal antibodies, and surface-enhanced Raman scattering (SERS) for the early stage diagnosis of TB and DF. Approaches will be detailed regarding the design and fabrication of chip-scale platforms, the selection of effective capture and tracer antibody pairs, the preparation of human sera, and the testing of assay accuracy via infected patient specimens. Comparisons to today’s gold standard measurements will also be presented and the potential merits of our approach critically assessed.

Keywords: Medical, Nanotechnology, Sample Preparation, Surface Enhanced Raman
Application Code: Biomedical
Methodology Code: Sensors
Metallic nanoparticles offer many opportunities in terms of detection including light scattering, surface plasmon resonance and surface enhanced resonance scattering (SERS). We are interested in the optical properties of metal nanoparticles and their potential application in a range of different biological studies. We can make use of the optical properties of nanoparticles in two ways.

1. The nanoparticle can act as an extrinsic label for a specific biomolecular target in the same way as a fluorescent label is used. The advantage of using the nanoparticle is its optical brightness (typically several orders of magnitude more than fluorophores) and the lack of background vibrational signals. Functionalisation of the nanoparticle with a specific targeting species such as an antibody or peptide aptamer allows this approach to be used in a wide range of studies including cell, tissue and in vivo analysis.

2. Nanoparticles can be designed to contain a specific recognition probe designed to cause a change in the aggregation status of the nanoparticles resulting in a discernible optical change when it interacts with its biomolecular target. This allows separation free analysis of specific biomolecular interactions and can be applied to a range of different probe/target interactions such as DNA-DNA, peptide-protein and sugar-protein.

We have been making use of nanoparticles in both of these approaches in conjunction with SERS which is an advanced vibrational spectroscopy. To demonstrate the applicability of the two different approaches examples will be given on the use of nanoparticles for cell imaging in two and three-dimensions, imaging of nanoparticles at centimetre depths through tissue and also their ability to report on biological molecules in vitro and in vivo.

**Keywords:** Biospectroscopy, Nanotechnology, Surface Enhanced Raman

**Application Code:** Nanotechnology

**Methodology Code:** Vibrational Spectroscopy
Targeting Protein-Protein Interactions

Protein-Protein Interactions Exploited Through Small Molecules in *Plasmodium Falciparum*

Malaria has been a human health concern for centuries, particularly in tropical and subtropical regions of the world. Nevertheless, our repertoire of medication to treat the disease has been very limited, and emerging resistance of the malaria parasite *Plasmodium* has further restricted the use of current medications. The most recent reports indicating artemisinin resistance in Cambodia are indeed alarming and underscore the critical importance of exploring novel pathways for interfering with the life cycle of the malaria parasite.

Utilizing a combination of computational as well as biophysical techniques we arrived at small molecules capable of interfering with the *Plasmodium* parasite. We will present data on two different protein-protein-interaction (PPI’s) and one ternary ligand-bound co-crystal structure. Small-molecules derived from our studies may represent useful tools for further dissecting and analyzing the autophagy and invasion pathway of *Plasmodium* as well as other apicomplexan species.

**Keywords:** Biosensors, Drug Discovery, Protein, X-ray Diffraction

**Application Code:** Biomedical

**Methodology Code:** Other (Specify)
Epigenetics classically defines the study of heritable phenotypes not genetically encoded in DNA. In cancer, epigenetic proteins are among the most promising and intently pursued targets in drug discovery. Already, inhibitors of DNA methyltransferases and histone deacetylases have demonstrated substantial clinical efficacy leading to regulatory approval for use in hematological malignancies. These events have triggered intense competition to develop inhibitors of chromatin-associated, gene regulatory complexes. Beyond drug development, chemical probes of gene regulatory proteins can provide insights into mechanisms of development and disease. We have approached the complex biochemistry of gene regulatory complexes with discovery chemistry, toward the goal of developing a chemical toolbox of novel, small-molecule modulators of chromatin structure and function. This presentation will describe recent research toward the development of drug-like inhibitors of epigenetic “readers” (BET bromodomains), “writers” (DOT1L and EZH2 lysine methyltransferases), and “erasers” (soft and selective HDAC inhibitors). In addition, research on the effect of unrestricted availability of chemical probes will be described, so as to describe the consequence of pursuing a more open-source model of drug discovery.

Abstract Text
Epigenetics classically defines the study of heritable phenotypes not genetically encoded in DNA. In cancer, epigenetic proteins are among the most promising and intently pursued targets in drug discovery. Already, inhibitors of DNA methyltransferases and histone deacetylases have demonstrated substantial clinical efficacy leading to regulatory approval for use in hematological malignancies. These events have triggered intense competition to develop inhibitors of chromatin-associated, gene regulatory complexes. Beyond drug development, chemical probes of gene regulatory proteins can provide insights into mechanisms of development and disease. We have approached the complex biochemistry of gene regulatory complexes with discovery chemistry, toward the goal of developing a chemical toolbox of novel, small-molecule modulators of chromatin structure and function. This presentation will describe recent research toward the development of drug-like inhibitors of epigenetic “readers” (BET bromodomains), “writers” (DOT1L and EZH2 lysine methyltransferases), and “erasers” (soft and selective HDAC inhibitors). In addition, research on the effect of unrestricted availability of chemical probes will be described, so as to describe the consequence of pursuing a more open-source model of drug discovery.

Keywords:
Chemical
Drug Discovery
Biomedical
Targeting Protein-Protein Interactions

Alpha-Helical Proteomimetics: Inhibition of Intracellular Protein-Protein Interactions via Direct Epitope Transfer from Proteins to Designed Small Molecules

Alpha-helices are central recognition elements in diverse protein-protein, protein-DNA, and protein-RNA interactions. We have developed a novel small molecule scaffold, based on tetrasubstituted tetrahydronaphthalenes, for the presentation of alpha-helical side chains along multiple turns of the alpha-helix on one protein alpha-helical face (Organic Letters 2013, 15, 4892-4895). The synthesis of the general scaffold proceeds rapidly from inexpensive starting materials and employs a novel, highly practical Mg(II)-catalyzed Friedel-Crafts epoxide cycloalkylation reaction. Each position on the scaffold may be differentiated after scaffold synthesis, generating a diversity-oriented approach to readily synthesize proteomimetics for different targets. The general scaffold is readily converted in 2-5 steps into mimics of a diverse range of recognition alpha-helices, including mimics of acidic activation domains, the estrogen receptor coactivator, and the androgen receptor coactivator. The synthetic approach to the nonpeptidic alpha-helical proteomimetics, based on the reaction of the scaffold alcohols with appropriate electrophiles, is general for the diverse incorporation of canonical and non-native protein side chains. This approach has been applied to develop a small molecule inhibitor of p53-MDM2. This designed small molecule, synthesized based only on known protein binding data without subsequent optimization, binds MDM2 with nanomolar affinity and inhibits the p53-MDM2 interaction in vitro and in human prostate cancer cells. The novel tetrasubstituted tetrahydronaphthalene scaffold allows the direct application of known structural data and rapid optimization of a small molecule mimic of a recognition alpha-helix using a readily modifiable organic scaffold, suggesting broad application in the development of small molecule inhibitors of alpha-helical protein-protein interactions.

Keywords: Amino Acids, Biomedical, Peptides, Protein
Application Code: Drug Discovery
Methodology Code: Biomedical
A very large number of biologically compelling drug targets are proteins whose biological function involves binding to other proteins. Historically, however, it has proven extremely difficult to identify small molecules that can bind to and inhibit protein-protein interaction (PPI) targets. This difficulty has led to the belief that many PPI targets are “undruggable”. In this talk I will describe the underlying reasons for the difficulty in targeting PPIs, and will discuss some potential strategies for overcoming these difficulties, involving the use of unconventional drug chemotypes such as synthetic macrocycles, covalent inhibitors, and peptide foldamers. I will illustrate these points by reference to our own efforts to identify small molecule antagonists of the PPI target NF-kappaB essential Modulator (NEMO).
In eukaryotes, intrinsically disordered (ID) proteins which lack stable secondary and tertiary structure in substantial regions (or throughout) are prevalent and exist as ensembles of rapidly fluctuating structures. These ID proteins are overrepresented in major disease pathways making them desirable targets for inhibition. Given their lack of a stable structure, however, these proteins were not considered druggable targets. We have determined that short, linear segments of ID proteins are sufficient for specific binding to small molecules and have demonstrated multiple binding sites in the dimerization regions of c-Myc and Id-2. The interaction between small molecules and disordered targets is amenable to structure-activity analysis and the generation of an effective pharmacophore model. We have conducted an alanine-scan of a binding site on c-Myc and find that both hydrophobic and hydrophilic amino-acids are crucial for binding, likely indicating that both hydrophobic interactions and hydrogen bonding contribute to the binding free-energy. To explain how multiple chemotypes bind the same short stretch of amino acids with apparent specificity, we provide a model based on different peptide conformations displaying different constellations of functional groups in order to recognize various chemotypes with chemical specificity. The model implies that while specific binding can and does occur, recognition of promiscuous binders is also likely.

**Keywords:** Drug Discovery, Drugs, Peptides, Protein

**Application Code:** Drug Discovery

**Methodology Code:** Molecular Spectroscopy
Proteins interact with other proteins and molecules as they function in biology. Highly specific and dynamic associations of cognate binding partners and modular protein domains govern cell growth, differentiation, and intercellular communication. Yet, compared to characterizing these components of life individually, elucidating structures of their assemblies is more challenging, potentially hampering efforts to understand their function and modulation.

Advanced mass spectrometry (MS) has capabilities to offer structural biologists layers of insight into the details of protein complexes. Mass measurements deliver information on stoichiometry of binding partners directly, even for multi-ligand hetero-complexes and molecular machines with masses well beyond 1 MDa. With electrospray ionization (ESI), MS can measure proteins and complexes from aqueous solution at near neutral pH, i.e., “native” MS. ESI’s gift for transforming solution-phase macromolecules into gas-phase ionized counterparts without disrupting covalent bonds and weak noncovalent interactions is key for applying MS to study protein complexes.

We have applied high resolution MS to yield structural information for large protein complexes. Tandem MS (MS/MS, or top-down MS) with collisionally activated dissociation (CAD) of noncovalent complexes resulting from electrostatic interactions can be used to probe ligand-binding sites (e.g., nucleotides, metals). Electron capture dissociation (ECD) can probe the binding sites of weakly bound ligands and the topology of large protein assemblies (e.g., complexes to 465 kDa). We are using ECD-Fourier transform ion cyclotron resonance (FT-ICR) MS to investigate the molecular action of compounds that prevent amyloid fibril formation in neurodegenerative diseases such as Alzheimer’s and Parkinson’s disease. We believe that structural methods based on MS will help advance our understanding of the functional role of proteins and biological macromolecules.

Keywords: Electrospray, Ion Cyclotron Resonance, Mass Spectrometry, Protein
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Core histones are heavily modified by multiple post-translational modifications (PTMs), generating histone code implicated in most chromatin-related cellular processes. Herein, we report an integrated C18 reversed phase liquid chromatography (RPLC) and weak cation exchange - hydrophilic interaction (WCX-HILIC) top down LC-MS platform for sensitive and comprehensive high throughput characterization of combinatorial histone PTMs.

Our results demonstrated that RPLC performs better for separating H2A and H2B proteoforms, which are mainly comprised of genetic variants and phosphorylated proteoforms, differing in hydrophobicity. Conversely, H4 has only two genetic isoforms and methylation and acetylation represent the majority of its PTMs. As these PTMs mainly occur in the polar tails, WCX-HILIC is a more suitable choice for separation of H4 proteoforms. H3 PTMs are significantly more complicated and have traditionally been analyzed with a WCX-HILIC approach. Surprisingly, C18 PRLC provided valuable insights into H3 proteoforms not observed using the WCX-HILIC. Employing different separation formats for different histone superfamilies facilitated identification of hundreds of unique histone proteoforms.

We then applied this improved workflow for mapping histones PTMs that regulate tamoxifen resistance in breast cancer to gain much needed information on the epigenetic control of this process. We used estrogen receptor, ER positive (MCF7) and ER negative (MDA-MB-231) cell lines to quantify changes in histone PTMs possibly associated with the differential tamoxifen sensitivity of these cells. We also examined how targeting a specific epigenetic mark, H3K27me3, affects combinatorial intramolecular PTMs co-occurring with modified H3K27. Preliminary results indicate that combinatorial histone PTMs are very dependent on the genetic background and targeting a single modification can have far reaching effects on additional PTMs.

Keywords: Biomedical, Mass Spectrometry, Protein, Proteomics
Application Code: Genomics, Proteomics and Other `Omics
Methodology Code: Liquid Chromatography/Mass Spectrometry
Heart failure (HF) remains a leading cause of mortality and morbidity in western countries. The cellular and molecular signaling mechanisms underlying cardiac dysfunction and the development of HF are complex and remain poorly understood. Aberrant protein post-translational modifications (PTMs) together with mutations and alternative splicing are increasingly recognized as important underlying mechanisms for HF. Therefore, a comprehensive study of cardiac protein PTMs is of paramount importance for understanding HF but presents tremendous challenges. Herein, we aim to develop and apply top-down mass spectrometry (MS) enabled cardiac proteomics technologies to study changes in cardiac protein modifications during disease progression to gain a better understanding of the molecular mechanism in HF.

Our research is highly interdisciplinary at the interface of chemistry, biology and medicine. On the technology front, we have made major progress in sample preparation for tissue proteomics and top-down MS/MS characterization of intact proteins. On the biology/medicine end, we made important contributions to myofilament biology/physiology and HF. We hypothesize that both extrinsic and intrinsic stresses trigger the molecular signaling processes that result in altered modifications to myofilaments leading to contractile dysfunction and eventually to HF. Toward such goals, we have: a) systematically analyzed thirty-six human clinical heart tissue samples and identified phosphorylation of cTnI, a key thin filament regulatory protein, as a candidate biomarker for chronic HF; and b) discovered augmented cTnI phosphorylation and identified the PKC-specific phosphorylation sites in spontaneously hypertensive rats. We are now working on mechanistically linking the proteomic changes to ex vivo and in vivo cardiac function as well as combining large animal (swine) model with human clinical studies to comprehensively assess synergistic changes of myofilaments in regulating cardiac contractility during left ventricular remodeling to HF.
We have established an analytical platform for top- and middle-down analysis of proteins, post-translational modifications (PTMs) and proteoforms (the combination of all sources of variation: PTMs, sequence variants, etc.). Our efforts integrate ultra high resolution 14.5T and 21T Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), electron transfer dissociation (ETD), specialized nanoflow chromatography, and both real-time, i.e. data dependent, and post-acquisition custom data analysis methods. Data analysis methods focus on isotopic fine structure under high-resolution mass spectrometric analysis, chromatographic time relationships between analytes, and the use of linear optimization methods to solve mixture models in multiple dimensions. We consider all the physico-chemical constraints simultaneously for identification and quantitation and allow for multiple interfering species that are only partially resolved in mass or time.

With these tools we observe statistically significant changes to distinct proteoforms in a variety of biological processes and in response to perturbations, both natural and engineered. Of particular interest to us is the role of specific combinations of post-translational modifications functioning in concert in biology, a question best addressed with top down mass spectrometry. These methods have been applied to a variety of problems. We have completed a quantitative analysis of the proteoforms of histone H2A and histone H2B, revealing the first protein level evidence for multiple histone variants as well as providing the most complete quantitative map of these key gene regulators. We have determined the PTMs and proteoforms of histone H4 that are present before and after the replication fork, yielding insights on how epigenetic information transits mitosis. We also have determined the protein sequence and proteoforms of the hyperphosphorylated heptarepeat C-terminal Domain of the RPB1 subunit of RNA Polymerase II.

**Keywords:** Bioanalytical, Biotechnology, Ion Cyclotron Resonance, Mass Spectrometry

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
Although the more widely used proteomics strategy has been peptide-based, unambiguous characterization of entire protein sequences and their post-translational modifications (PTMs) is often best achieved through the analysis of intact proteins. Previously, the field of Top Down proteomics has lagged behind Bottom Up in terms of high-throughput and comprehensive analysis. This was attributed to underdevelopment of separations, MS instrumentation and bioinformatics. Recently, our lab has developed a platform for the large scale use of multiplexed 2D separations coupled to capillary LC-FTMS to achieve unprecedented proteome coverage for proteomics based on whole proteins. Given the complexity of the human proteome, the new separation platform integrates protein fractionation by charge, mass and hydrophobicity. An analysis of HeLa and H1299 cells (+/- DNA damage) using this platform resulted in approximately 50 fractions covering 4 < pl < 10 and MW up to 100 kDa in a total separation time of 3.5 hours. Online detection and top down protein identification resulted from scan events that included both MS1 scans using an FTICR or Orbitrap, with fragmentation scans using HCD, ETD, and CID methods. Data were identified using ProSight software and the Q-value approach of Storey and Tibshirani. With six days of automated instrument time, 580 unique accession numbers were identified. Compilation of identifications between untreated and DNA damaged samples led to a total of >1000 Top Down identifications in several cell lines.

Keywords: Mass Spectrometry, Proteomics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Mass Spectrometry
Protein aggregation remains one of the most confounding post translational modifications that can occur to a protein therapeutic in that it is difficult to detect via standard analytical techniques. This is due to the fact that most aggregation is non-covalent in nature and is easily disrupted by most methods (reversed phase chromatography, mass spectrometry, etc.). Detecting and quantitating aggregated proteins requires methods non-denaturing separation methods like gel filtration chromatography (GFC) which separate proteins based on their apparent size in solution. While this method is an isocratic HPLC method and much less complex than other chromatographic methods, there are several method optimization parameters that are required for separating protein and their aggregates with high resolution and recovery. Using GFC for protein aggregation determination will be discussed as well as other analytical techniques (MALS, Analytical Ultracentrifugation, PAGE) for determining protein aggregation state.

Keywords: Biopharmaceutical, Characterization, Chromatography, HPLC Columns
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Analytical Challenges Facing the Characterization of Targeted Monoclonal Antibody-Based Therapies

New analytical strategies are required to characterize the current tidal wave of targeted mAbs, some of which include: bispecific mAbs, bispecific diabodies, and bispecific single chain antibody molecules. In general the therapeutic dosing of these molecules is very low, (ug/ml concentrations). To adequately characterize these molecules, adaptations to current methods or innovative approaches are necessary.

Keywords: Biopharmaceutical, Characterization
Application Code: Other (Specify)
Methodology Code: Liquid Chromatography
Advances in Protein and Peptide Separations

Strategies for Increasing the Sensitivity and Selectivity of LC/MS/MS Techniques

LC/MSMS has long been the analytical gold standard for high sensitivity quantitation of complex samples. The universality of modern ultra pressure chromatography coupled to electrospray ionization mass spectrometry provides the most generally applicable starting point for many laboratory experiments across a wide range of analyte classes. Naturally the wider use of LC/MS/MS across many industries has uncovered many instances where greater selectivity or sensitivity is needed for quantitative assays. Therapeutic peptide quantitation as well as clinical diagnostic biomarker quantitation assays are commonly sensitivity and/or selectivity limited. Traditionally MRM or MS/MS type of experiments have been viewed as the most sensitive/selective approaches for quantitation. Occasionally however, nominal mass and accurate mass MS/MS experiment cannot overcome isobaric background and positional isomers that can complicate chromatograms. Several approaches are available to increase the sensitivity and selectivity of LCMSMS assays.

Commonly, gains in selectivity are accompanied with losses/tradeoffs in signal. To counter this a common strategy is to simply increase the raw ion sampling of the source/interface of mass analyzer as well as increase the transmission of ions in the mass analyzer region. The greater number of ions can often enable many approaches such as high resolution mass spectrometry, MS/MS/MS ("MS3" or "MS cubed") as well as orthogonal techniques based on differential mobility. These techniques have been proven to reduce background levels and increase overall signal-to-noise of analytes. Another area of improvement is in the area of chromatographic selectivity as well as sample preparation. Commonly solid phase extraction (SPE) can introduce some level of selectivity to reduce background interferences. In many cases, SPE can increase signal to noise, throughput and robustness of LCMS assays.

Keywords: Liquid Chromatography, Mass Spectrometry, Peptides, Quantitative
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Liquid Chromatography/Mass Spectrometry
Methionine Oxidation in an antibody Fc domain has the potential to reduce binding to the FcRn receptor and impact pharmacokinetics. As a result, it is a Critical Quality Attribute (CQA) that needs to be monitored during process characterization/process validation (PC/PV) studies. In order to measure Fc oxidation with high throughput, we developed a sample preparation method using the FabRICATOR enzyme, which cleaves an antibody to produce an Fc fragment. The Fc fragment is then analyzed by a RP-UHPLC method, which has a 15 minute run time. In this study, monoclonal antibody oxidation levels are compared using the conventional peptide map (206 minutes) versus our new FabRICATOR UHPLC assay (15 minutes).

Keywords: Characterization, Enzyme Assays, HPLC, Process Analytical Chemistry

Application Code: Process Analytical Chemistry

Methodology Code: Liquid Chromatography
The utility of LC/MS is mitigated by the fact that analysts are rarely presented with a sample in a suitable form for injection and there are few robust alternatives to time consuming benchtop methods. As a potential solution to the sample preparation bottleneck this presentation will teach the details of a high throughput, column-based, automated approach. The use of affinity selection, immobilized enzymes, on-line desalting and integrated reverse phase separation will be described and their limitations balanced in regards to their utility.

Keywords: Automation, Bioanalytical, Biopharmaceutical, Characterization
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Liquid Chromatography/Mass Spectrometry
This presentation will provide an overview of high throughput food analysis. A definition of ‘high throughput’ will be discussed and different analytical methodologies examined in that context. Different types and classes of food contamination will be highlighted. Biological screening methods as well as instrumental methods for high throughput analysis will be described, focusing particularly on new developments in the area of LC-MS and LC-MS/MS. The presentation is intended to provide a general context for understanding the subsequent, more specific presentations that follow in the session.
High Throughput Analysis for Food Safety and Cosmetics

Rapid and Simultaneous Determination of Harmful Chemicals in Nail Products by Gas Chromatography-Tandem Mass Spectrometry

Many nail products may contain potentially harmful ingredients such as toluene, methylpyrrolidone, benzophenone-1, and diethylene glycol dimethacrylate. A rapid and sensitive gas chromatography-tandem mass spectrometry (GC-MS/MS) method has been developed and validated to determine the level of these harmful chemicals in nail products. In this procedure, test portions were extracted with acetone followed by vortexing, sonication, centrifugation, and filtration. Benzophenone-1 was derivatized to a less polar compound using N,O-Bis(trimethylsilyl)trifluoroacetamide during the extraction procedure to make it amenable to GC analysis. The four compounds were quantified by GC-MS/MS in the electron ionization mode. Four corresponding isotopically labeled analogues were selected as internal standards, which were added at the beginning of the sample preparation and used to correct for recovery and matrix effects. GC-MS/MS, derivatization of benzophenone-1 and extraction conditions were optimized to minimize serious interference for the quantification of the analytes. The results show that the linear range was 100 – 50,000 ng/mL for toluene and benzophenone-1, respectively. Results of methylpyrrolidone and diethylene glycol dimethacrylate showed a linear range from 100 to 5,000 ng/mL. This method has been applied to screen commercial nail products for the above four compounds. The average recoveries for these compounds, at three spiking levels, ranged from 98% to 109%. In a limited survey of 20 nail products, the values for toluene ranged from 3.9 to 229,000 [micro]g/g in 17 products. Methylpyrrolidone ranged from 17 to 102 [micro]g/g in 2 products. Diethylene glycol dimethacrylate ranged from 1.6 to 35 [micro]g/g in 4 products. Benzophenone-1 ranged from 5.6 to 2,371 [micro]g/g in 10 products. This is the first time that a GC-MS/MS method with dynamic selected reaction monitoring and confirmation of analytes has been used to simultaneously determine these four compounds in nail products.

Keywords: Capillary GC, Cosmetic, Gas Chromatography/Mass Spectrometry
Application Code: Other (Specify)
Methodology Code: Gas Chromatography/Mass Spectrometry
**Session Title**: High Throughput Analysis for Food Safety and Cosmetics  
**Abstract Title**: Antibiotic Residue Detection by LC/MS/MS in Food  

**Primary Author**: Angela Carlson  
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**Co-Author(s)**:  

**Abstract Text**

Bioassays for detection of antibiotic residues are time consuming and cannot always identify the specific residue and quantity present. Detection of many classes of antibiotics in various food matrices can be achieved by utilizing solid phase extraction (SPE) cleanup and analysis by High Performance Liquid Chromatography with tandem mass spectrophotometry (LC/MSMS). Using LC/MSMS allows for high-throughput screening while still providing identification of specific residues and quantitation at part per billion (ppb) levels.

**Keywords**: Chromatography, Liquid Chromatography/Mass Spectroscopy, Quadrupole MS

**Application Code**: Food Science

**Methodology Code**: Liquid Chromatography/Mass Spectrometry
Impact of Chronic Ethanol Consumption on Metabolite Profiles of Liver in Mice: A Time Course Study

Approximately 1 in 3 adult Americans has abnormal liver enzymes, indicating fatty liver disease. To investigate the impact of chronic ethanol consumption on metabolite profiles of mouse liver, a time course study was performed by feeding the male C57BL/6N mice with different diets, including a modified Lieber-DeCarli alcohol liquid diet. At the end of each feeding time point, mice were anesthetized, and liver tissue was collected from each mouse. Metabolites were extracted from mouse liver using a mixture solvent methanol-water (v:v=4:1). The extracted metabolites were derivatized using N-(tert-Butyldimethylsilyl)-N-methyl-trifluoroacetamide (MTBSTFA). The derivatized samples were analyzed on a comprehensive two-dimensional gas chromatography time-of-flight mass spectrometer. The experimental data were first analyzed using the instrument software ChromaTOF for peak picking and compound identification, followed by MetPP software for retention index matching, alignment, normalization, quantification, and time course analysis.

Keywords: Bioanalytical, Bioinformatics, GC-MS, Metabolomics
Application Code: High-Throughput Chemical Analysis
Methodology Code: Gas Chromatography/Mass Spectrometry
High Throughput Analysis for Food Safety and Cosmetics

A Mass Spectroscopic Fingerprinting Method for Authentication and Quality Assessment of Scutellaria lateriflora Based Dietary Supplements

Abstract Text
Scutellaria lateriflora, commonly known as skullcap, is used as an ingredient in numerous herbal products. However, it has been occasionally adulterated/contaminated with Teucrium canadense and/or Teucrium chamaedrys, commonly known as germander, due to the morphological similarities between the two genera. The latter contains hepatotoxic diterpenes. Despite the potential hepatotoxicity introduced by germander contamination, analytical methodologies for the authentication and quality assessment Scutellaria lateriflora based dietary supplements have not been reported. In this study, a flow injection electrospray ionization/mass spectrometry (ESI/MS) fingerprinting method in combination with principal component analysis (PCA) was used to survey Scutellaria lateriflora based dietary supplements sold in the US.

Keywords: Chemometrics, Mass Spectrometry, Natural Products
Application Code: High-Throughput Chemical Analysis
Methodology Code: Liquid Chromatography/Mass Spectrometry
This paper presents an application of ultra-high performance liquid chromatography (UHPLC) and micro flow liquid chromatography (MFLC) coupled with electrospray ionization (ESI) quadrupole Orbitrap high resolution mass spectrometry (Q-Orbitrap) for determination of ~ 500 pesticide residues in fruits and vegetables. MFLC/ESI Q-Orbitrap was explored its applicability for analysis of pesticides using 1/1000 dilution concept to minimize matrix effects and increase sensitivity. MFLC/ESI Q-Orbitrap can provide low ppt sensitivity and is suitable for this 1/1000 dilution approach and still can achieve the most of regulatory requirement on pesticide analysis in nine matrices without any sample cleanup requirement. It is a truly high through put approach for sample analysis in the laboratory and further approved through PCA analysis for the feasibility of this approach.

Abstract Text

Keywords: Food Science, High Throughput Chemical Analysis, Liquid Chromatography/Mass Spectroscopy, Mass
Application Code: Food Science
Methodology Code: Liquid Chromatography/Mass Spectrometry
High Throughput Analysis for Food Safety and Cosmetics

Improving Identification of Pesticides Using Atmospheric Pressure Gas Chromatography Coupled with Mass Spectrometry

Successful testing of pesticides in food requires techniques allowing for fast and reliable determination of residues, and the absence thereof. Mass spectrometry offers residue chemists the ability to discriminate against thousands of peaks, identifying trace level residues in a variety of food matrices. Electron impact (EI) ionization in gas chromatography mass spectrometry provides highly specific data for many trace level pesticides. Analytes that produce mass fragments unique to the compound deliver confidence when reviewing mass spectral data. However, compounds exist that do not produce mass fragments indicative of the analyte. As the size of the fragment decreases, so does selectivity and sensitivity, resulting in difficult data interpretation, particularly at sub part per billion levels. Fragments too small to be detected may result in false negatives. Additionally, co-eluting matrix interferences present in sample extracts obscure targeted mass peaks in the chromatography, contributing to false positive determinations.

Atmospheric pressure ionization gas chromatography (APGC) provides a softer ionization of the analyte, preserving the molecular ion for better identification. This technique coupled with accurate mass quadrupole time of flight mass spectrometry, produces both low and high energy spectra. Pesticides evaluated for APGC included pyrethroids, historically difficult to interpret with EI fragmentation. Lower mass fragments were primarily produced in the high energy spectra, whereas in most cases low energy spectra successfully generated the molecular ions for improved identification. Results will be presented including signal to noise of the molecular ions and key fragments for complex matrices including flaxseed and dried soybean. This technique will offer more confidence in a reliable result, aiding in faster, higher throughput for data analysis.

Keywords: Food Science, Gas Chromatography/Mass Spectrometry, Pesticides, Time of Flight MS
Application Code: Food Science
Methodology Code: Gas Chromatography/Mass Spectrometry
Doing more with less is a common mantra in today’s business environment, prompting a push for employees to work smarter. Advances in predictive software tools provide scientists the opportunity to streamline their processes while simultaneously improving the quality of their work product through the application of statistics in a quality-by-design approach.

We have incorporated the use of two software tools to streamline and standardize HPLC method development activities from lead optimization up through commercialization.

One of the softwares relies on a database built using a set of generic method conditions, using in-house compounds chosen to maximize structural diversity while maintaining a representative charge state distribution. When presented with novel molecules, this software performs a search for similar structures and generates a correlation equation based on calculated physiochemical properties. These equations are then used to rank the generic methods according to their predicted ability to separate the compounds of interest. This is a simple way to improve the quality of methods for early stage projects, when knowledge and resources can be limited.

Once more robust, stability-indicating methods are needed to support pre-clinical and clinical studies, The other software provides an automated platform for statistics-based method development and enables scientists to rigorously explore and define an operating space, within which the method meets all user-defined criteria.

Together, these predictive softwares can be powerful resources in the modern chromatographer’s toolbox.

Keywords: HPLC, Method Development, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Robustness of liquid chromatographic methods can often be difficult to assess. Traditional approaches which involve one factor at a time may not predict real world scenarios. Robustness pitfalls are therefore one of the major challenges of method development.

This talk will discuss an approach to method development which not only effectively assesses robustness, but also optimizes the method for the most robust method possible. The approach is a multivariate one, whereby Design of Experiments (DOE) software manages the study. The talk defines the concept of "Method Robustness Optimization," and follows up with a case study to show how it's implemented.
Size Exclusion Chromatography (SEC) is one of the most common and widely used liquid chromatography (LC) based methods to determine the size and purity of the molecule. With advancement in technology it becomes imperative to develop a high throughput and robust SEC method. Fusion AE is a QbD aligned LC method development software. It is capable of creating automated experimentation which follows FDA/ICH guidance and can communicate with Chromatographic Data Systems (CDS) directly. It’s patented statistical tools like Trend Responses™ and Robustness Simulator™ which help in providing best solution for a robust method.

A UHPLC based SEC method, targeting for a higher throughput as compared to traditional HPLC based method, was developed using Screening and Optimization modules of Fusion AE software. Based on the critical quality parameters of the method, a design space was generated by Fusion AE’s QbD Visualization Graphics and the most robust method was selected for future analysis.

Keywords: Liquid Chromatography, Particle Size and Distribution, Statistical Data Analysis
Application Code: High-Throughput Chemical Analysis
Methodology Code: Liquid Chromatography
Since the regulatory introduction of Quality by Design (QbD) principles for pharmaceutical products a decade ago, more pharmaceutical companies are adopting the principles of Quality by Design (QbD) for pharmaceutical development and manufacturing. Described in ICH Q8, Q9 and Q10 guidance documents, QbD enables enhanced process understanding, and a more systematic and scientific approach to development, Analogous to QbD for product, QbD may also be applied to analytical methods, by defining the required analytical performance and systematically relating how the technique and method parameters affecting analytical performance. Examples will be presented demonstrating our experience of achieving the appropriate level of method understanding by applying a risk-based multivariate approach via statistical design of experiments (DOE) or mechanistic models. This approach has better defined an approved method operating region which can then be validated. Furthermore, we’ve adopted an approach of monitoring the performance of the method over time so that it can be periodically be evaluated from method design stage throughout its lifecycle of use.

Keywords: Data Analysis, Quality
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
FusionAE (S-Matrix Corp., Eureka, CA) experimental design and data analysis software was instrumental in completing the analytical method development for screening kits of labeled reference standards. NSK-A is a mixture of twelve 13C and Deuterium labeled amino acids and NSK-B is a mixture of Carnitine and its seven acyl derivatives, which are labeled with Deuterium atoms. Both serve as calibrators for testing by Mass Spectrometry. All analytical methods to confirm identity and quantity of the compounds in these standards have to be validated for their intended purpose. FusionAE software provided a very useful and flexible template for prospective validation of a variety of analytical methods needed for these products. The analytical methodology included quantitative NMR for substrate purity/content and HPLC for product content, as well as GC/MS for isotopic enrichment of environmental standards produced under ISO 17025 (Guide 25) guidance.

In addition to these validations, the method development module of FusionAE software was used for NSK-B HPLC method optimization to increase signal strength and separation. This constituted a critical method improvement, as the concentration of carnitines in NSK-B standard is very low (close to the detection limit of the Evaporative Light Scattering detector used in this method) and they are structurally very similar. Use of the method development module of the FusionAE software minimized the number of runs needed to complete this task and made it less complicated.

Keywords: Amino Acids, GC-MS, HPLC, Other Hyphenated Techniques
Application Code: General Interest
Methodology Code: Liquid Chromatography/Mass Spectrometry
QbD Based Development of Analytical Methods for Product Characterization, Release, and Stability Study

Pursuing the "Perfect" HPLC Method Using Quality by Design

When applied to High Performance Liquid Chromatography (HPLC) method development, Quality by Design provides information on how controllable instrument parameters affect chromatographic performance. This presentation will discuss the practical application of QbD and its impact on the quality of the HPLC method.

Primary Author: Joseph A. Turpin
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Abstract Text

Keywords: HPLC, Quality
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
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, particularly after the publication of ICH Q8-Q11 documents. The paper will focus on key lessons learned in implementation of QbD approach for method development through validation and transfer. Multi-factorial analysis as opposed to OFAT approach truly helps in developing a robust and optimized method. The process may initially appear cumbersome, but it saves time and money. The QbD based software is obviously not a substitute to having expertise in chromatography. Advanced level background in statistics is also very useful. The design space established through the QbD based method development could be useful in modifying the method post-approval.

**Keywords:** Chromatography, Liquid Chromatography, Statistical Data Analysis

**Application Code:** Pharmaceutical

**Methodology Code:** Computers, Modeling and Simulation
Here we present the development of a SERS-based assay for the in-situ detection of multiple fungal targets (Candida glabrata, Candida albicans, Candida krusei, Aspergillus terreus and Aspergillus fumigatus). All the fungi identified have shown to pose a serious health risk to immunocompromised individuals, therefore prompt and accurate diagnosis is paramount to ensure the correct antifungal treatment is used. SERS has readily been shown to have multiplexing capabilities but the transferal of the techniques attributes to a closed system presents a huge challenge. The conjugation of sequence specific DNA to nanoparticles offers extremely accurate discrimination of targets but the application to samples containing multiple targets, is not without its difficulties. One of the initial problems was the ineffective synthesis of nanoparticle-oligonucleotide conjugates, it will be described here how this was overcome using a pH-modified buffer, which greatly increased the stability of the probes, drastically reduced the synthesis time and also increased yield. The issue of migrating dye tags will also be examined along with the effectiveness of using fluorescent dye tags inserted directly into the oligonucleotide strands.

**Keywords:** Bioanalytical, Biospectroscopy, Nanotechnology, Surface Enhanced Raman

**Application Code:** Bioanalytical

**Methodology Code:** Biospectroscopy
This work presents gold microhole arrays as a highly sensitive plasmonic material for SPR sensing. We already reported that these microhole arrays improve sensitivity for the detection of IgG by SPR in Kretschmann configuration by three fold. These substrates support both propagative and localised plasmonic modes generating a strong electromagnetic field at its surface. This electromagnetic field could be used to get metal enhanced fluorescence (MEF) signals of labelled proteins in order to improve their detection.

By tuning the microhole arrays physical properties, we can tweak its plasmonic wavelength and thus adjust its coupling to a fluorophore or a laser. We have coated different microhole substrates with Rhodamine 6G to compare their coupling with the fluorophore. We observed a significant enhancement from our substrates when compared to a continuous gold film. By using fluorescence microscopy, we also assessed the localisation of the fluorescence on the substrates. We found out that by tuning the thickness of gold, it is possible possible to change the localisation of the plasmonic enhancement from the terraces to the holes areas. In addition, these structures can be used as SERS substrates where one can observe ring-like hotspots around the rim of the holes.

A simultaneous detection of correlated fluorescence and SPR signals will lead to more reliable and sensible immunoassays and with our approach, this test can be done within an hour.

Keywords: Bioanalytical, Fluorescence, Nanotechnology, Surface Enhanced Raman
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Bioanalytical Spectroscopy

Ultrasensitive Detection of Dyes and Proteins by Surface-Enhanced Raman Spectroscopy (SERS) in Capillary Electrophoresis (CE)

We demonstrate ultrafast and highly sensitive surface-enhanced Raman spectroscopy (SERS) detection of analytes in flow following capillary electrophoresis (CE) separation using a custom-built flow cell. The system consists of a fused silica capillary pinned to the bottom of a flow channel that delivers the sample into the detection volume. Hydrodynamic focusing generated by a sheath flow inside the flow channel is used to direct the analyte molecules onto a planar SERS substrate where increased interactions with the intensive electric field near the substrate surface results in improved sensitivity. The potential of implementing CE and SERS is demonstrated for the separation and subsequent spectroscopic identification of dyes and proteins. Sequential and high throughput flow detection of separated analyte molecules is achieved at nanomolar concentrations using a 50 ms acquisition without significant memory effect or fouling of the SERS substrate. The implementation of this robust, sensitive, and reproducible online CE-SERS detector is shown to provide highly detailed structural information following chromatographic separations for a variety of analytes with high biochemical relevance.

Keywords: Vibrational Spectroscopy, Capillary Electrophoresis, Lab-on-a-Chip/Microfluidics, Surface Enhanced Raman Spectroscopy

Application Code: Bioanalytical

Methodology Code: Vibrational Spectroscopy
G-protein coupled receptors (GPCRs) regulate several biochemical signaling pathways related to many disease states. Thus, measuring ligand-GPCR interactions is an important method in drug discovery, with nearly 40% of known drug targets being GPCRs. Unfortunately, a direct measurement of ligand-GPCR interactions in heterogeneous environments is challenging. To overcome this challenge, we developed an assay that utilizes ion channel-coupled GPCR receptors (ICCRs) expressed in a mammalian cell vector. ICCRs are fusion proteins that combine GPCRs with ion channels (ICs) to form ligand-gated ICs activated by ligand-GPCRs-interactions. Expression of the ICCR in cellular models generates electrically active cells that respond directly to the ligand-ICCR binding event, with less dependence on downstream signaling cascades. In order to characterize functional ICCRs, various ICCRs were expressed in HEK293 cells and verified by indirect immunohistochemistry. ICCR responses were evaluated in a 96-well plate assay composed of transfected HEK293 cells with agonist, antagonist and toxins. Pancreatic beta cells (INS-1) containing native ICs and non-transfected HEK293 cells were tested as controls. Positive response was observed by the decrease of DIBAC4(3) fluorescence intensity and the increase of Fluor-4 AM florescence intensity indicated membrane depolarization and increased intracellular Ca2+ concentration, respectively, in transfected HEK293 cells treated with GPCRs agonists, and toxins. Moreover, the response was attenuated upon addition of a GPCR agonist in transfected HEK293 cells. This 96-well plate assay provides a faster and less laborious alternative to patch clamp methods for evaluating ICCR activity and should significantly increase the capability to analyze complex drug mixtures in a high-throughput fashion.
Bioanalytical Spectroscopy

NIR Dyes As Substrates: New Approach to Determine Enzymatic Activity

Near-Infrared absorbing and fluorescent carbocyanines are increasingly used in analytical chemistry. The enzymatic activity of alkanesulfonate monooxygenase is frequently monitored by using n-alkanesulfonate compounds as substrates by determining n-alkanealdehyde products. Due to the relative volatility of the n-alkanealdehydes quantification of the product can be cumbersome. Accordingly our research group evaluated the use of NIR carbocyanines containing n-alkylsulfonate moieties attached to the heterocyclic nitrogen as alkanesulfonate monooxygenase substrate. The resulting n-alkylaldehyde substituted carbocyanine product is non-volatile and can easily be identified and quantified using standard analytical tools such as CE or spectroscopy. In this study we first characterized a new class sulfonated heptamethine and pentamethine dyes for this purpose. Both symmetrical and asymmetrical carbocyanines were evaluated. The influence of the length of the alkyl chain and the length of the polymethine chain on the enzymatic activity was studied for both mono and bis substituted carbocyanines. During these studies these dyes were successfully used in vitro photo-reduced riboflavin mononucleotide (FMN) with a glucose/glucose-oxygenase oxygen scavenging system. The reduced FMN serves as a key substrate in the enzymatic desulfonation. NIR laser induced fluorescence (LIF) detected CE was utilized to detect the sulfonated and de-sulfonated dyes. To further simplify the detection of the alkyaldehyde substituted carbocyanine product of the enzymatic reaction we utilized fluorescence changes that can be observed when carbocyanines bind to biomolecules such as serum albumins. Due to the changes in the hydrophobicity of the carbocyanine dyes upon desulfonation spectral properties of the albumin bound dye change which can be utilized to quantify the product of the enzymatic reaction. This new approach can simplify enzymatic activity measurements as no volatile products are generated.

Keywords: Bioanalytical, Capillary Electrophoresis, Enzyme Assays, Molecular Spectroscopy
Application Code: Bioanalytical
Methodology Code: Biospectroscopy
Receptors are key components of the cell membrane that coordinate the passage of signals into and out of cells. Mechanisms for controlling this flow of information include receptor clustering and rearrangement of membrane organization. We are elucidating the role of other membrane proteins, extracellular ligand, and intracellular proteins in altering receptor clustering and diffusion using several fluorescence microscopy techniques. Our work provides vital information on the molecular mechanism of receptor function through altered dynamics and membrane organization.

Clustering is measured using fluorescence resonance energy transfer (FRET) and stimulated emission depletion imaging (STED). In order to measure membrane structure and dynamics, we have developed a STED instrument for fluorescence lifetime imaging with 40-nm spatial resolution, which is below the diffraction limit of light. Diffusion is measured using fluorescence recovery after photobleaching (FRAP) and single particle tracking (SPT) with ligand-labeled quantum dots. Selected membrane proteins that we study include integrins, receptor for advanced glycation end products, focal adhesion kinase and epidermal growth factor receptor, while selected cytoplasmic proteins that we study include paxillin, vinculin, actin and focal adhesion kinase. Among our interesting findings, we have determined that other membrane proteins do not significantly alter the percentage of mobile integrins, but significantly constrain integrin diffusion; the role of cytoplasmic proteins in altering integrin clustering depends on the concentration of extracellular ligand available for binding; and increasing integrins' ligand affinity reduces the population of mobile integrins while also reducing the diffusion coefficient of integrins that remain mobile. The fluorescence microscopy tools that we develop are suited to measurements of the diffusion and clustering of numerous membrane proteins.

Abstract Text

Keywords: Bioanalytical, Fluorescence, Imaging, Instrumentation
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Artificial lipid bilayers are promising candidates for biocompatible coatings, drug delivery vehicles, and membrane-protein-based biosensor matrices. The fragility of lipid bilayers formed from natural lipids can be overcome by using polymerizable lipids to extend the lifetime and robustness of membranes. We found the function of the ion channel gramicidin is enhanced in mixtures of poly(lipid) and diphyPC, but is lost when in pure poly(lipid). Because gramicidin monomers diffuse through the membrane, these findings suggest lipid membrane fluidity may be important to membrane-protein function. We hypothesize that polymerized bilayers have reduced fluidity in comparison to unpolymerized bilayers, while mixtures of non-polymerizable and poly(lipids) have intermediate fluidity.

Fluorescence recovery after photobleaching (FRAP) measurements were completed to determine diffusion coefficients and mobile fractions of polymerizable dienoylPC planar supported lipid bilayers (PSLBs) on glass. The fluidity of unpolymerized and UV-polymerized PSLBs was measured. Polymerization temperatures above and below the phase transition of the unpolymerized lipid were employed in pure systems. With the exception of mono-DnPC, UV-polymerization decreased the diffusion coefficients of all polymerizable lipids. UV-polymerization of bis-substituted lipids resulted in < 100% mobility. In diphyPC/bis-SorbPC mixtures, it was found that UV-polymerization did not significantly effect diffusion until < 50% of the lipid was polymerizable.

This research is supported by the NIH and NSF.
Trypanosoma brucei is the causative agent of African trypanosomiasis. Together with other kinetoplastid parasites, these microorganisms cause ~30 million cases of disease a year. Combating these protozoan pathogens presents an ongoing challenge due to the lack of techniques for quantifying complex and dynamic biological parameters in live cells. Specifically, the conditions within kinetoplastid glycosomes are of significant importance because they are the center of metabolism and are vital for cell survival.

Here we report an adaptation of a fluorescein-tagged peroxisomal targeting sequence (F-PTS1, acetyl-CKGGAL) for ratiometric pH quantification in live Trypanosoma brucei (T. brucei). When added to cells, these fluorescent peptides are internalized within vesicular structures and can be visible after 60 min. Using F-PTS we are able to observe the pH conditions inside glycosomes in response to starvation conditions. In the absence of glucose or proline metabolites, the glycosome exhibits an acidification from pH 7.4 to 6.8. This pH regulation is found to be independent from cytosolic pH and requires a source of Na+. Glycosomes were also found to be more resistant to external pH changes than the cytosol, suggested that regulation of Glycosomal pH is different and independent from cytosol pH regulation. Furthermore, pH regulation is suggested to work by an active process as cells depleted of ATP were unable to properly regulate pH. siRNA and inhibitor studies suggest that certain proteins are responsible for glycosomal pH regulation.

Keywords: Bioanalytical, Fluorescence, Microscopy
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Capillary electrophoresis (CE) is well-suited for the separation of peptides and proteins though analyte-wall interactions can hinder separation performance. Surface coatings have been used to improve CE performance by minimizing these interactions. In addition to reducing adsorption, surface coatings dictate the direction and magnitude of the electroosmotic flow (EOF). To resolve complex mixtures of peptides and proteins it is important to use a surface coating with the appropriate EOF. Analyte electrophoretic mobility can vary greatly across peptides and intact proteins. Ideally, a surface coating method would provide a range of EOF values from which to choose such that separation performance could be optimized for the analytes of interest. In addition to minimizing surface interactions and providing the appropriate EOF it is paramount, for peptide and protein separations, that surface coatings for CE are compatible with electrospray ionization-mass spectrometry (ESI-MS). This work describes a surface coating method resulting in a covalent, MS-compatible surface with a range of anodic EOF magnitudes at low pH. This coating method was applied to integrated microfluidic devices, fabricated from glass, designed for CE-ESI applications. CE-ESI microchips coated in this way produced efficient separations of both peptides and intact proteins (> 1 million theoretical plates per meter). The peak capacity and resolution of these CE-MS experiments was improved by choosing surface coating reagents to generate the appropriate EOF as dictated by the electrophoretic mobilities of the analytes.

Keywords: Capillary Electrophoresis, Electrospray, Lab-on-a-Chip/Microfluidics, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
The use of a self-assembling nanogel material composed of phospholipid additives is used for the separation of DNA biomarkers. Because this material displays thermally reversible morphologies and viscosities it is being investigated for use with tunable DNA sieving. For applications such as human identification where the target DNA is under 500 base pairs, a Q2.5 10% hydration preparation has been optimized for Ogsten sieving in this range. Additional studies with temperature variations suggest this Ogsten sieving regime can be tuned for larger DNA fragments. By manipulating the nanogel material with temperature gradients during separation, better resolution can be achieved for DNA fragments up to 10,000 base pairs. The material is also being investigated for use with single stranded DNA applications using urea in the separation matrix. This material is based upon work supported by the National Science Foundation under award number CHE-1212537 and #1003907.
As for carbohydrate analyses by capillary electrophoresis (CE), we focused on the affinity CE (ACE) analysis using quinolineboronic acids (QBAs) which form complexes with cis-diol compounds like most carbohydrates. In a background solution (BGS) containing QBAs, the complexation provides the variations of both the fluorescence intensity and the apparent electrophoretic mobility of the complex from those of native carbohydrates, so that both the label-free fluorescence detection and the selective separation of carbohydrates are expected. As a result, nine carbohydrates, including sorbitol, mannitol, galactitol, catechol, galactonic acid, arabitol, xylitol, meso-erythritol, and mannose, were separated with a BGS containing 5-isoQBA, which indicated the successful detection and separation of these carbohydrates without any derivatization.

As for another ACE analysis, a novel capillary partially filled with an affinity ligand-encapsulated hydrogel using sodium alginate (SA) was prepared. Alginate hydrogel (AH) can be easily formed by adding a calcium ion into an SA solution and encapsulate proteins with maintaining their affinity. An SA solution containing avidin was partially injected into the capillary filled with a BGS containing calcium dichloride, then an appropriate voltage was applied to introduce the calcium ion into the SA solution zone for the hydrogelation. The encapsulation of avidin by the formed AH was expected. To clarify the formation of the avidin-encapsulated hydrogel, a sample solution containing biotin was analyzed in the prepared capillary. In repeated analyses without refreshing the hydrogel, biotin was not detected due to a strong interaction with avidin at the first two runs, whereas it appeared after the third run. These results suggested the saturation of the binding site of the encapsulated avidin with the introduced biotin. Consequently, affinity ligands can be encapsulated successfully by the AH with maintaining their affinity.

Keywords: Bioanalytical, Capillary Electrophoresis, Carbohydrates, Method Development
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
This work aims to understand the molecular mechanism(s) for chiral separations using bile salt aggregates as chiral selectors and model racemic compounds such as 1,1’-binaphthyl-2-2’-diyl hydrogen phosphate (BNDHP) and 1,1’-bi-2-naphthol (BN) as guest analytes. The chiral selectivity of bile molecule micelles was studied using micellar electrokinetic capillary chromatography (MEKC) and \(^{1}\)H nuclear magnetic resonance (NMR). A primary critical micelle concentration (cmc) of cholate was reported by BN and BNDHP \(^{1}\)H NMR chemical shifts between 10-14 mM and 15-20 mM, respectively. Notably MEKC chiral separations of racemic BN and BNDHP are achieved above 17 and 20 mM, respectively. NMR studies of higher concentrations of cholate in the presence of BN and BNDHP have reported a secondary cmc around 30 mM which corresponds to the observed decrease in resolving power of the MEKC separation (difference in stereo-selective mobility, \([\text{sub}_{ep}/\text{sub}]) of racemic BN and BNDHP above 30 mM. A van’t Hoff relationship was observed using the \([\text{sub}_{ep}/\text{sub}]) values of the individual BN isomers as proxies for the equilibrium constant, which suggests thermodynamic insights into micelle formation. The apparent enthalpy changes for both isomers are endothermic and equivalent from 17 to 25 mM cholate. However, there is a sharp increase in the apparent enthalpy changes between 25 and 30 mM cholate further suggesting a secondary aggregation event. Further understanding of the dynamics of bile micelle solutions has been informed by analyte concentration, pH and counterion identity.

This work is supported by NSF-RUI Grant (#CHE-1153052).

Keywords: Capillary Electrophoresis, Chiral Separations, NMR
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Capillary Electrophoresis: New Approaches for Bioanalytical Applications

Capillary Electrophoretic Separations with Post Capillary Droplet Segmentation and Sample Capture

With liquid chromatography separation systems the ability to capture, manipulate, and further analyze a sample, or components thereof, following a separation is a relatively straightforward process. This affords greater capabilities to liquid chromatography based analyses than are possible with current capillary electrophoretic separations. Due to the nature of capillary electrophoretic separations the sample zones at the outlet of the capillary are inherently small and these zones are transferred directly into vastly larger buffer reservoirs, where the dilution effectively loses the sample entirely. Consequently, the collection of samples from capillary electrophoretic samples for further manipulation is rarely performed.

This work will present our approach to the collection of separated sample components from the outlet of a capillary electrophoretic separation. We have managed to successfully encapsulate the outlet flow of the capillary electrophoretic separation in a continuously flowing stream of liquid perfluorocarbon, resulting in nanoliter volume sized droplets of effluent and sample which can be further manipulated and analyzed while suffering minimal dilution or diffusion effects. Crucially, this has been accomplished not through the use of complexly fabricated microfluidic systems, but rather with conventional chromatographic tubing and unions, allowing for the widespread adoption of this tool for further exploration by any research group. We will present the fabrication of the droplet formation system, the considerations for the design and its application to the separation of simple biochemical samples.

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Capillary Electrophoresis: New Approaches for Bioanalytical Applications

Understanding In-Line Mixing and Stacking Dynamics with EMMA Using the Jaffe Reaction

Electrophoretically Mediated Microanalysis (EMMA) has proven useful for carrying out in-line chemical and biochemical assays. However, the local dynamics of (i) in-line electrophoretic mixing of reagents and (ii) post-reaction stacking of products are difficult to predict. This work involves experimentation as well as computer simulation to better understand the complex ionic migration inherent to the heterogeneous and rapidly developing EMMA system. The small molecule Jaffe reaction, which is clinically used for determination of creatinine in biofluids, is used as a model system. The EMMA approach for this system is faster than the traditional methodology, and generates much less waste. Effective reagent overlap and stacking of the in-line product lead to optimal EMMA sensitivity, which is important for small molecule EMMA applications that employ UV-VIS detection. Characterization of the in-line mixing and stacking events is aided by the use of Simul 5.0 customized to allow for the kinetics of the in-line reaction. Matching the ionic strength in the reagent zones is found to provide optimal robustness in the mixing step, and the addition of a concentrated (> 0.1 M) plug of highly mobile hydroxide ions is found to be helpful for stacking of the in-line product. In addition, the length of the hydroxide plug can be used to adjust the timing of the stacking event such that it corresponds with the time that the product zone migrates past the detection window. In all, Simul is shown to be a powerful tool to help understand the migration dynamics during EMMA experiments.

Keywords: Bioanalytical, Capillary Electrophoresis, Software
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Capillary isoelectric focusing (cIEF) can provide both powerful enrichment and separation of proteins and peptides based on the analyte’s isoelectric point (pI). However, on-line coupling of cIEF with ESI-MS for analysis of protein digests is challenging due to the presence of ampholytes, which generate strong interfering signals in the same m/z range as tryptic digests. We have recently demonstrated that amino acids can act as ampholytes to create the pH gradient in cIEF while generating very low background signals in the m/z range for tryptic peptides. We observed that the background signal intensity generated by the amino acids was a factor of 30 lower than that generated by commercial ampholytes, which was valuable in the study of low abundance peptides by ESI-MS/MS. Protein quantification is valuable in understanding changes in biological systems accompanying development, differentiation, and disease. We report the application of amino acids based cIEF for quantitative analysis of a complex proteome. Biological duplicates were generated from PC12 cells at days 0, 3, 7, and 12 following treatment with nerve growth factor. These biological duplicates were digested with trypsin, labeled using eight-plex iTRAQ chemistry, and pooled. The pooled peptides were separated into 25 fractions using RPLC. Technical duplicates of each fraction were analyzed by amino acid cIEF-ESI-MS with an electrokinetically-pumped sheath-flow nanospray interface. This HPLC-cIEF-ESI-MS/MS approach identified 835 protein groups and produced 2,329 unique peptides IDs. The biological duplicates were analyzed in parallel using conventional SCX–RPLC-ESI-MS/MS which generated 1,369 protein groups and 3,494 unique peptide IDs. For protein quantitation, 96 and 198 differentially expressed proteins were obtained with RPLC-cEIF and SCX-RPLC, respectively. The combined set identified 231 proteins. Protein expression changes measured by RPLC-cEIF and SCX-RPLC were highly correlated.
During the past decade there has been a considerable increase in the abuse of illegal and prescription drugs, the latter enhanced by internet sales. One of the greatest dangers of drug use is combining it with driving. More than 11% of drivers in a 2007 National Highway Traffic Safety Administration’s (NHTSA) roadside survey tested positive for illicit drugs, while 18% of drivers killed in accidents tested positive for illicit, prescription or over-the-counter drugs according to a 2009 NHTSA survey. Consequently, there is a need for a noninvasive roadside drug testing device, similar to the breathalyzers used by law enforcement officials to measure and estimate the blood alcohol concentration levels of impaired drivers. In an effort to meet this need we have been investigating the ability of surface-enhanced Raman spectroscopy (SERS) to detect and identify numerous drugs in saliva at ng/mL concentrations within 10 minutes. Identification is provided by matching measured spectra to a SERS library comprised of over 150 different drugs, each of which possess a unique spectrum. Trace detection is provided by gold nanoparticles trapped within a porous glass matrix that generate SERS. Speed is provided by a syringe driven sample system that extracts the drugs from saliva AND provides SERS-activity. Spectral collection is provided by a portable Raman analyzer. Here we describe successful measurement of representative illicit, prescribed, and over-the-counter drugs in saliva by SERS, with a focus on cocaine.

Keywords: Clinical/Toxicology, Drugs, Surface Enhanced Raman, Ultratrace Analysis
Application Code: Clinical/Toxicology
Methodology Code: Vibrational Spectroscopy
A standardized and sensitive quantitative method has been developed to extract and analyze a suite of hydrophobic organic toxins from a wide variety of matrices. Volatile and (at ambient) nonvolatile analytes were extracted from biological fluids, water samples, powdered food extracts, and soils simultaneously by dual stir bar sorptive extraction (D-SBSE). Compounds are thermally desorbed and chromatographically analyzed using gas chromatography/mass spectrometry. Direct quantification is performed using isotope dilution mass spectrometry (IDMS), without the use of external calibration curves. This novel method was developed to be robust and broad in scope to replace many of the outdated solid-phase extraction technique still being used by industrial, commercial, and environmental laboratories. The combined techniques of D-SBSE-GC-IDMS provide greater method efficiency, sensitivity, precision, and accuracy over existing methods. Method performance is compared against currently accepted extraction techniques, such as solid-phase extraction, solid-phase microextraction, and sonication-induced extraction. Method validation was performed for each analyte and matrix by the extraction and IDMS quantification of a certified reference standard spiked into each matrix. This method was developed to extract and quantify a specified suite of organic toxins representing a wide range of hydrophobicity and volatility in a single extraction step. However, toxins of interest may differ by region or application. Therefore, future research seeks to incorporate analyst-defined toxins, while maintaining the performance and validation of this method.

Keywords: Environmental, Extraction, Gas Chromatography/Mass Spectrometry, Solid Phase Extraction
Application Code: Clinical/Toxicology
Methodology Code: Gas Chromatography/Mass Spectrometry
Real-time multimodal monitoring of the injured human brain is currently an area of great interests, as it offers the possibility of the real-time monitoring and treatment of patients. We have previously shown that measurements of the brain electrocorticography (ECoG) and neurochemical changes (glucose, lactate and potassium) by the online microdialysis technique, which allows the detection of transient adverse events called spreading depolarisation (SD). We now are building all of these monitoring modalities into a single clinical instrument. This abstract describes the design of the instrument and relevant measurement electronics of the system.

The instrument has three electronic monitoring sections: low-noise and low-cost 8-channel ECoG signal detection system, which is able to detect the signal in the uV level; 4-channel Ion Selective Electrode (ISE) monitoring unit, which is used for measuring local potassium concentrations in the brain cells and it has mV resolution; 4 potentiostats with different gains which allow researchers to measure the extra-low current from local glucose/lactate changes, ranging from 1pA to hundreds nA. All these electronic monitoring sections and relevant data transmission are controlled by a field-programmable gate array (FPGA), allowing real-time on-device control and the optimisation of the performance.

Preliminary results will be presented from the human brain.

The project is funded by Wellcome Trust/DOH HICF grant.

Keywords: Clinical Chemistry, Electrochemistry, Ion Selective Electrodes, PCB's
Application Code: Clinical/Toxicology
Methodology Code: Electrochemistry
Persistent Organic Pollutants (POPs) are known to be resistant to degradation in the environment; therefore, bioaccumulation is a concern in human health. Polycyclic Aromatic Hydrocarbons (PAHs) are a category of POPs created through incomplete combustion reactions. Due to their carcinogenic and mutagenic properties, bioaccumulation is a major issue. Spring Creek, a nationally recognized fishing stream, has been shown to contain many PAHs both in and around the creek. Sediment samples from this area are the samples being investigated. When PAHs are present, they present a distinct signature. Automotive exhaust is a common source for PAHs, but the fingerprints found in the Spring Creek show evidence of at least one more source of PAHs beyond automotive emissions. Because PAHs in the sediment samples are from both natural and anthropological sources, PAH ratios will be employed in the identification of possible sources. Ratios between parent PAH groups and their alkylated counterparts will also be used to identify whether the source may be petrogenic or pyrogenic.

A “library” of PAH fingerprints will be developed by collecting samples from various possible PAH sources. This study will show the development of a methodology to analyze and identify possible sources of a PAH contamination. Many of the samples in this study have very complex signatures. Extensive cleanup is necessary to analyze these samples. This presentation will address the needs and development of the extraction and cleanup procedures prior to instrumental analysis. Additionally, GC-MS will be utilized in this study. Due to the high molecular weight of some of these compounds, unusual demands are placed upon the chromatograph. As a result, various sample introduction techniques are employed, and these will be compared and contrasted. Finally, a quadrupole mass analyzer is being employed, and pros and cons of this detector will also be discussed.

Keywords: Environmental/Soils, GC-MS, PAH, Quadrupole MS
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
A novel polymeric ionic liquid (PIL)/multi-walled carbon nanotube (MWCNT)-based sorbent coating was prepared via thermal initiated on-fiber copolymerization for solid-phase microextraction (SPME) analysis. Polycyclic aromatic hydrocarbons (PAHs) were selected as model analytes to evaluate the extraction efficiency and analytical performance of the coating. PIL-based sorbent coatings containing different amount of MWCNTs were prepared for a comparison study. PIL/MWCNT-based sorbent coatings demonstrate higher extraction efficiency and selectivity for the extraction of PAHs compared with neat PIL-based sorbent coating and a commercial polydimethylsyloxane (PDMS) coating. Insight into the extraction mechanism of the PIL/MWCNT-based sorbent coating will be discussed. The film thickness of all studied sorbent coatings were determined via scanning electron microscope (SEM) and the analyte-to-coating partition coefficients were estimated. The limits of detection (LOD) for all PAHs were determined to be in the sub ng L-1 range using the PIL/MWCNT-based sorbent coatings.
Polycyclic aromatic hydrocarbons (PAHs) are widely studied in the environmental matrices, such as air, water, soil and sediment due to their toxicity, mutagenicity and carcinogenicity. Due to this, the environmental agencies of developed countries listed sixteen PAH as priority pollutants. Few countries have limits for these compounds for ambient air, only for emissions from stationary and mobile sources and occupational areas, and there are several studies, more specifically for the 16 priority PAHs and very little for the alkyl PAH. These compounds are more abundant, more persistent and frequently more toxic than the non-alkylated PAHs, and toxicity increases with the number of alkyl substitutions on the aromatic ring. In this study, it was developed a method for analysis of PAHs and alkyl PAHs using GC-MS and large injection volume injection coupled with program temperature vaporization, which allow limits of detection bellow 1.0 ng L⁻¹. Several variables were tested, as injection volume, injection velocity, injector initial temperature, duration of the solvent split and others. The method was evaluated in samples from particulate matter from engines emissions using standard diesel, commercial diesel and biodiesel B20. Samples were collected on a dynamometer bench for diesel engine cycle and the results were in the range of 0.5 to 96.9 ng mL⁻¹ and indicated that diesel/biodiesel have a significant contribution to the formation of PAH and alkyl PAH.

Keywords: Biofuels, Environmental/Air, GC-MS, PAH
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Common methods to analyze semi-volatiles are EPA Methods 525, 625, and 8270. All of these methods have proven problematic because of the wide variety of compounds that are included in these methods. The compounds include very active compounds like acids, bases, and even active pesticides. The poor performance of these compounds often affects method performance by increasing the limits of detection (LOD) for the active compounds, and even limiting the life of the hardware by eventually failing QC performance requirements.

Many of these problems can be mitigated by having an inert flow path for active compounds. The items that have the largest impact on active compound performance are the liner and the column. This work explores the affects of different liners and columns and how these affect the performance of active compounds in semi-volatile analyses like phenols, amines, and pesticides.
Controlled substances such as cocaine, heroin, methamphetamine and marijuana, are drugs commonly seized by law enforcement officers and routinely analyzed by forensic laboratories. Synthetic drugs such as bath salts and cannabinoids, as well as, other naturally occurring substances have increased in popularity as chemical stimulants and opiate substitutes. This study focuses on the botanicals Catha Edulis, a drought-tolerant Middle-eastern shrub containing cathinone, and Mitragyna Speciosa, an indigenous tree of Southeast Asia. Use of these materials affects the central nervous system and produces adverse physiological effects. The wide concentration range and chemical diversity of components in these samples are an analytical challenge. Utilization of gas chromatography - high performance time of flight mass spectrometry (GC-HRT) is a practical choice for the analysis of these substances. The instrument’s high resolving power minimizes background interferences, while its excellent mass accuracy values allow for confident elemental formula determination. In addition, complete and high quality spectral data allow for library searching against accurate as well as, nominal mass libraries (e.g., NIST). Data acquisition is comprehensive, robust and can be used for both targeted and non-targeted analyses.

Keywords: Forensics, Time of Flight MS
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography/Mass Spectrometry
The consumption of mushrooms containing hallucinogenic substances for intoxication is a common problem around the world. Adverse physiological effects such as tachycardia and hypertension have been attributed to the indole derivatives psilocin and psilocybin present in several types of mushrooms; however, other bioactive compounds (e.g., amphetamines) may contribute or be entirely responsible for some of these effects. Analysis is complicated by the diversity and large quantities of fatty acids, esters, alcohols, sugars and other compounds present in mushrooms. In addition, some compounds such as psilocybin are labile and may decompose under certain instrumental conditions. In this study, several sampling techniques were used to maximize the number of mushroom constituents extracted and identified. The utilization of GCxGC high performance time of flight mass spectrometry (TOFMS) is a practical choice for analysis of complex materials such as mushrooms. Data acquisition was comprehensive, robust, and useful for both targeted and non-targeted analyses. Single acquisition, high resolution, accurate mass data (< 1ppm) was probed multiple times for biologically active compounds. The increased chromatographic resolution provided by GCxGC, coupled with the high resolving power of TOFMS minimized background interferences, and excellent mass accuracy values allowed for confident elemental formula determinations. In addition, complete and high quality spectral data facilitated library searches against both accurate and nominal mass libraries (e.g., NIST 2011, Wiley 9).

Keywords: Forensic Chemistry, GC-MS, Sample Preparation, Time of Flight MS
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography/Mass Spectrometry
Decomposition is a very complex process that is not very well understood. Several studies have investigated the accumulation of volatile organic compounds (VOCs) produced during the early stages of human decomposition. However, our knowledge of the chemistry of decomposition is limited because of poor reproducibility and comparability within and between published studies and the difficulty in obtaining human corpses for decomposition studies. Several studies have shown that pig (Porcus) carcasses develop similar decomposition profiles to those of human corpses. Therefore, a comprehensive investigation (with larger and consistent sample sizes) of VOC profiles using pig carcasses is in progress, which will provide important information about the unknown chemical composition of death.

Specifically, this study focuses on the most prevalent VOCs that are detected during each stage of decomposition. VOCs will be collected, identified and quantified from decaying pig carcasses using passive headspace collection via solid phase microextraction (SPME) fibers. The VOCs will then be analyzed and quantified using a gas chromatograph-mass spectrometer (GC-MS) instrument. As stated previously, pigs will be used as models for humans and the pigs will be euthanized via captive bolt as to ensure no chemical interferences. This study is broken into two phases; an indoor phase and an outdoor phase. Decomposition in an indoor enclosure will eliminate many of the uncontrolled factors that affect decomposition. This will provide information as to whether a consistent VOC profile is obtainable. Decomposition outdoors will take into account insect activity and the various weather factors such as rainfall and temperature.

Keywords: Air, Forensics, Gas Chromatography/Mass Spectrometry, SPME
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography/Mass Spectrometry
The shocking increase in the number of “meth houses” and “mobile meth labs” in rural parts of the United States is becoming a prevalent problem. Defiant Technologies, located in Albuquerque, NM, has developed a possible tool for law enforcement. The Canary-3 is a handheld, battery-powered micro gas chromatograph for vapor-phase detection of semi-volatile organics like methamphetamine and pseudoephedrine. The Canary-3’s small size and low power operation is accomplished through the use of three specialized micro-fabricated components: a micro pre-concentrator coated with a proprietary sol gel, a 2.5 meter long micro-GC column, and a surface acoustic wave detector. No specialty gases are required for operation since the system uses ambient air for the carrier gas. A typical analysis can be completed in less than seven minutes. The Canary-3 will display the chemical name and concentration on its LCD screen and an on-board micro-SD card will log every chromatogram collected in the field. The Canary-3 includes software that enables the user to create calibrations for their target analytes, import data from the Canary, and view data live if desired. We have demonstrated collection, separation, and detection of methamphetamine and pseudoephedrine in the vapor phase using the Canary-3. Each sample was prepared using premixed standards in an air sample bag and introduced directly into the inlet of the Canary-3 for analysis. A limit of detection was calculated for both pseudoephedrine and methamphetamine. In this presentation, we will present the technology underpinning the Canary-3, its use as a portable GC/SAW field instrument for semi-volatiles, and its application with methamphetamine/pseudoephedrine.

Keywords: Gas Chromatography, GC, Lab-on-a-Chip/Microfluidics, Portable Instruments
Application Code: Homeland Security/Forensics
Methodology Code: Portable Instruments
In order to efficiently respond to terror threats, there is an increasing interest in rapid analysis of off-normal events, including real-time data obtained from in-situ sensors. We are investigating the possibility of quickly obtaining chemical information about the fireball which results from explosions in hopes that this will enable us to identify the constituents of the original explosive device.

In this presentation, we will first outline the operational situations in which we are interested, discuss previous efforts in this field, and identify promising spectroscopic techniques. We will also present the initial results from using open-path FTIR to monitor the fireball and after effects from traditional explosives. The presentation will conclude with a discussion of the implications of the initial field tests and future modifications of the test procedures to achieve rapid field identification of explosive devices.

The work has been sponsored by the Defense Threat Reduction Agency (DTRA).

Keywords: Characterization, Environmental/Air, Forensic Chemistry
Application Code: Homeland Security/Forensics
Methodology Code: Vibrational Spectroscopy
Arson investigation is one of the most complex challenges encountered by forensic chemists. Fire weathers ignitable liquids (ILs) and pyrolyzes substrates, greatly increasing the variability and complexity of the already uncontrolled and complex mixture of matrix and IL. Headspace extraction of debris samples followed by gas chromatography-mass spectrometry (GC-MS) is the common approach for fire investigation. The most time-consuming step for the analysts is data interpretation. Our group has previously demonstrated an approach for the automated construction and optimization of chemometric models that have successfully classified both simulated and casework debris samples on the basis of gasoline content.

While these previous models (casework and simulated) were individually successful, the model based on simulated debris could not be used for the classification of casework samples. This is not an issue for large laboratories handling many samples and for the identification of gasoline: the most commonly used IL. However, in order to obtain training sets for chemometric models to classify less common ILs in a timely fashion, debris must be simulated. In this contribution, we present a comparison of chemometric models based on both casework debris and debris simulated using a new approach. Gasoline is used as the model IL to validate the simulation approach, which is then extended to other ILs.
Identification of ignitable liquid residue in fire debris is complicated by the presence of pyrolysis products and error rates for the determination are generally not known. The purpose of this research is to establish statistically valid error rates for the determination of fire debris as positive or negative for ignitable liquid residue. The average mass spectrum across the chromatographic profile, referred to as the Total Ion Spectrum (TIS), has been shown to be useful in the identification of individual ignitable liquids and ASTM E1618 classes of ignitable liquids. These results are supported agglomerative hierarchical cluster analysis and self-organizing feature maps (SOFM). The TIS have also been shown to be useful in classifying fire debris residue as positive or negative for ignitable liquid residue using hard classification methods of LDA and QDA, and soft classification by SIMCA. Fire debris samples from laboratory-scale and field test burns were also used to test the models. The optimal model’s true positive rates for fire debris samples were 90.2%, 82.9% and 95.2% for LDA, QDA and SIMCA, respectively. False positive rates were 10.0%, 1.3% and 18.3% for LDA, QDA and SIMCA, respectively. This work was supported by the National Institute of Justice, Office of Justice Programs, Award 2009-DN-BX-K227. The content of this publication does not necessarily reflect the position or the policy of the Government, and no official endorsement should be inferred.
We report the development of an inexpensive and disposable colorimetric paper sensor array for the detection and discrimination of five explosives – triacetone triperoxide (TATP), hexamethylene triperoxide diamine (HMTD), 4-amino-2-nitrophenol (4A2NP), nitrobenzene (NB), and picric acid (PA). The colorimetric sensor comprised a disposable paper array fabricated using a wax printer and three reagents (KI, creatinine, and aniline) that produced a unique pattern for each explosive based on chemical interactions between the explosive species and the chemical reagents. The analytes were discriminated from one another as per the colour change profiles, which were readily distinguishable after 15 min, using hierarchical clustering analysis (HCA) and principal component analysis (PCA); there were no misclassifications in any of the trials conducted. The colorimetric pattern values were extracted using a smartphone (RGB values from the center of the spot from the “before” and “after” images), custom-made software and a closed chamber to circumvent the illumination problems commonly find in other paper devices methodologies. Additionally, quantitative analysis were performance using an approach based on the colorimetric response of the spots expressed using the Euclidian distance (ED) versus the concentration of each explosive and the limits of detection were estimated as 0.4 µg (4A2NP), 0.2 mg (NB), 2.3 µg (PA), 1.0 µg (HMTD) and 0.2 µg (TATP). Financial support: FAPESP, CAPES and CNPq.
Abstract Text

HPLC is one of the most widely applied analytical techniques. It can serve as a powerful separation technique for chemical mixtures consisting of a wide range of compounds including isomers. HPLC with its choice of detection techniques can separate the analytes but provides little information about what a compound might be. The photodiode array can provide some information about peak purity or presence of coeluting peaks but can only provide identification by comparing to standards in the same mobile phase. Adding a mass detector to a HPLC-PDA system can provide information for peak identification, for recognizing coeluting chromatographic peaks and for confirming peak purity. A mass detector, however, cannot provide complete sample characterization by itself. It may not prove useful in distinguishing isomers or compounds that either poorly ionize or do not ionize under the selected conditions. Components present in trace levels may go undetected against a more abundant background and ion suppression may affect detection of coeluting peaks. We have employed orthogonal detection techniques to help fully characterize a USP tablet following the monograph guidelines for sample preparation. The combination of the chromatographic configurations including the HPLC system with a diode array detector, a mass detector and software tools to combine data from these orthogonal detectors provides information to enable a more thorough characterization of the sample.

Keywords: Characterization, Chromatography, Detection, Mass Spectrometry
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Sulfite is a widely used food preservative which can cause severe allergic reactions in sensitive individuals. As a result, the U.S. FDA requires that sulfite be listed on the label of any food product containing more than 10 ppm sulfite (measured as sulfur dioxide). Currently, the optimized Monier-Williams method (AOAC Official Method #990.28) is the most common method for determining sulfite concentrations in foods. However, this method is tedious and can give false positive or negative results. This research describes the development of a LC-MS/MS method for determining sulfite in various food matrices including dried fruits, dried and canned vegetables, canned and frozen seafood, and juices. The matrix is extracted with a buffered formaldehyde solution converting free and reversibly bound sulfite to the stable formaldehyde adduct, hydroxymethylsulfonate (HMS). Extracts are prepared for injection using a C18 SPE cartridge to remove any lipophilic compounds. HMS is then separated from other matrix components using hydrophilic interaction chromatography (HILIC) and detected by negative ion electrospray tandem mass spectrometry using multiple reaction monitoring (MRM). The method was validated at 5 concentrations in 15 food matrices according to the FDA Foods Program Guidelines for Chemical Methods. In these validation studies, spiked recoveries ranging from 84-115% were observed for dried fruit, jam, vinegar, and juice products. This improved, rapid and selective method will allow better enforcement of the sulfite labeling requirements, which will enable sensitive individuals to avoid inadvertently ingesting sulfite.

Keywords: Food Science, Liquid Chromatography/Mass Spectroscopy, Method Development, Tandem Mass Spectrometry
Glutathione (GSH) is a water-soluble tripeptide composed of the amino acids glutamine, cysteine, and glycine. The thiol group is a potent reducing agent, rendering GSH the most abundant intracellular small molecule thiol, reaching millimolar concentrations in some tissues. GSH is an important antioxidant in detoxifying a variety of electrophilic compounds and peroxides. The importance of GSH is evident by the widespread utility in plants, mammals and fungi. A deficiency of GSH puts the cell at risk for oxidative damage.

The current USP method for impurity analysis uses ion-pair chromatography. Problems with using ion-pairing include long column equilibration times, irreproducible retention, and devoting a column to a single analysis once the column is used with the ion pair reagent. An improved alternative to the USP and EP methods using LC/MS/MS to identify and quantify the impurities is presented using no ion pairing in the analysis.

A glutathione certified reference material was used in this study. MRM transitions for the determination of ions for the parent and impurities were determined. Two transitions were used for each ion. Structures for the major impurities were confirmed and structures for other smaller impurities are proposed. In addition to improving the specificity of the method with MS/MS detection, two additional goals were to minimize analysis time and eliminate the need for ion pairing reagents that are not compatible with MS. A series of LC columns with varying chemistries were screened to give the best resolution and the shortest analysis time for glutathione and its impurities.

Keywords: Chromatography, HPLC Columns, Liquid Chromatography/Mass Spectroscopy, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
The characteristics of the components within a sample determine the complexity of chromatographic methods development. Often, two of the largest problems are managing the range of chromatographic behavior found in a typical formulation and, second, ensuring peak identification and quantitation for a range of components with different detection properties. The analysis of a common nutraceutical supplement containing glucosamine HCl and vitamin D3 serves as an example. This formulated product has active ingredients that are exceedingly polar and exceedingly non-polar. Additionally, these ingredients span the range from strong UV absorbance to complete transparency. Highly polar sample components, like glucosamine, will not retain well in reversed phase (RP) mode. Hydrophilic interaction chromatography (HILIC) has been found to be a more effective approach. The exclusive nature of the mobile phases, wash solvents and columns associated with HILIC makes running both modes a challenge to methods development. When components have no UV response, sample detection also becomes a challenge. Most often, a mass spectrometer (MS) is added to augment UV detection. Adding a MS, as well as managing the different data acquisition and processing needs of the detectors, further complicates method development. A chromatographic system performing both RP and HILIC modes in a single set-up, with integrated optical and mass spectral detection is used to develop methods for the analysis of glucosamine and vitamin D3 from a nutraceutical supplement. The system software allowed automation of instrument operation and harmonized the acquisition and processing of both the optical and mass spectral data.

Abstract Text

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Date: Tuesday, March 04, 2014 - Afternoon
Time: 02:30 PM
Room: S502b

Session Title
Liquid Chromatography/Mass Spectrometry: Pharmaceutical and Environmental Applications

Abstract Title
Automated Multimodal Chromatographic Method Development Integrating Complementary Optical and Mass Spectral Detection

Keywords: Chromatography, Liquid Chromatography/Mass Spectroscopy, Method Development
Application Code: Other (Specify)
Methodology Code: Liquid Chromatography/Mass Spectrometry
When developing methods for an unknown sample, long separation methods with high resolution are developed to ensure that all components of the sample are separated. This approach has two desired outcomes. First, all the components are separated with no coelutions, and, second, all important impurities are detected. In reality, neither objective is met with certainty. We have approached this complication by combining two orthogonal detectors that are sensitive to different physical principles. This facilitates recognizing coelutions and for more completely detecting the components of the sample. This system includes the tools necessary for adjusting the separations if any coelutions are recognized. With complete separation and orthogonal detection, it is possible to construct libraries describing all the sample components and impurities by their retention time and spectral properties. In the course of analyzing an authentic sample, any peak can then be confirmed as a member of this library and the amount can be compared to specified limits. This workflow will be applied to an assay, based on an USP method of extracted tablet where many excipients and related compounds are present. The library will be used to confirm or exclude the presence of impurities. This will be based on confirming the mass, retention time and UV spectra of these impurities.
The occurrence of 1,3-dimethylamylamine (1,3-DMAA) concentrations in geranium plants is currently important from a regulatory perspective. Following the death of two U.S. soldiers who had ingested dietary supplements containing 1,3-DMAA, the United States Food and Drug Administration issued a warning letter to the supplement manufacturers contesting the manufacturers’ claims that 1,3-DMAA is eligible to be labeled as a dietary ingredient under the Dietary Supplement Health and Education Act of 1994. These actions have led to multiple, conflicting reports confirming or denying the presence of 1,3-DMAA in geranium plants.

Recently, 1,3-DMAA concentrations and its isomer counterpart, 1,4-DMAA, were determined at the 10 – 350 ng/g level in geranium plants harvested in China. This work was followed by a complimentary derivation method in which the DMAA species were reacted with (-)-1-(9-fluorenyl)ethyl chloroformate (FLEC) in order to form six diastereomer products. This (-)-FLEC analysis lacked product stability and chromatographic resolution of the stereoisomers was not ideal; however the analysis determined that 1,4-DMAA was racemic within the error of analysis.

An alternative chiral derivatizing agent (CDA), Mosher’s acid chloride, will be presented for 1,3-DMAA and 1,4-DMAA stereoisomer analysis using HPLC with tandem MS. This CDA contains the same functional group reaction site as the (-)-FLEC reagent but is a much smaller compound, allowing for less steric hindrance in the product. Reaction parameter, separation and MS optimization will be presented along with stereoisomer analysis of 1,3-DMAA and 1,4-DMAA in geranium plants.

Keywords: Chiral Separations, Derivatization, Environmental/Biological Samples, Liquid Chromatography/Mass S
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
Liquid chromatography is used as an analytical technique to provide qualitative and quantitative sample composition. Development of a suitable chromatographic method can be a complex process that exploits many physical and chemical parameters. With changes in chromatographic variables, the sample components may change their elution order. This adds uncertainty to systematic method development in that comparisons of different conditions may not accurately track each sample component and coelutions may go unrecognized. A chromatographic system for separations development should provide both efficient exploration of separation space and complete detection for assessment of the results. For the separation, the system includes an automated screening of different columns, as has been routine for some time. Selectivity changes derived from mobile phase manipulation have been harder to define and automate. It is cumbersome and time-consuming to prepare the combinations of solvents and pH/ionic strength modifiers. Instrumental functions have been combined with solvent management algorithms to permit continuous adjustment of pH while using different organic solvents. These functions are combined with column screening and variations in physical operating parameters to fully explore separation space. These experiments, however, require a way to track the individual components within the chromatogram. Here, the combination of photodiode array and mass detectors is considered as an approach to complete sample characterization. The complementary data sets from the two detectors are combined using new software tools that can aid peak tracking and the assessment of peak homogeneity. The combination of mobile phase manipulation with information-rich component identification and peak tracking in a unified UPLC system makes chromatographic method development more complete and efficient.

**Keywords:** Chromatography, HPLC Detection, Liquid Chromatography, Liquid Chromatography/Mass Spectrometry

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Oxcarbazepine belongs to a group of medicines called anticonvulsants and mood stabilizing drugs. A reversed-phase liquid chromatographic method was developed and validated to assay Oxcarbazepine in the raw material and determine impurities/degradants. A Thermo, BDS HYPERSIL C18 (4.6 × 250 mm, 5 μm) column was used with UV-Vis detector at 257 nm. The optimum conditions were concluded under column temperature 25οC, injection volume of 15 μL, flow rate at 1.0 mL/minute. The mobile phase consisted of Potassium Phosphate Monobasic at pH 2.9 and Acetonitrile with the isocratic elution 23:77% ACN: buffer for 20 minutes.

A series of forced degradation studies were carried out in which sample solutions of raw material were subjected to different stress conditions of acid and base hydrolysis, hydrogen peroxide oxidation, heat and UV light following the ICH guidelines. Under the optimum separation conditions, the Oxcarbazepine active ingredient was separated from the potential impurities/degradants, which were separated from each other as well. The correlation coefficients for the linearity of Oxcarbazepine raw material and the impurity/degradants were 0.9997 and 0.9989, respectively. The percent recovery of Oxcarbazepine and the impurities/degradants were in the range of 98% to 102% and 90% to 110%, respectively. The detection limit for impurities/degradants was found 0.3 ppm with the limit of quantification of 0.75 ppm. The developed method was validated for specificity, robustness, linearity, accuracy, precision, limit of detection and limit of quantitation. The method was found to be simple, accurate and precise for the assay of Oxcarbazepine in the raw material as well as determination of impurities/degradants.
Microfluidic methods have distinct advantages for gradient formation including low consumption of reagents, precise control of reactions, and high throughput fabrication of devices. We have designed a microfluidic diffusion diluter to create stable chemical gradients across an array of cell cultures. In this study we exploited diffusional mixing in a center channel, allowing a reagent and buffer to mix as a function of distance and to diffuse into culture channels. The device enabled concentration based studies to be conducted at 256 different concentrations across individual, low shear cell cultures. Mass transport into and out of the culture channels occurs mainly via diffusion with a small convection component. COMSOL multiphysics was used to create theoretical model for the chip. Chemical gradient generation was demonstrated using fluorescien dye mixing with Phosphate Saline Buffer controlling flow rates. Numerical simulations were used to validate the gradient generation in microfluidic diffusion diluter. The computational results compared well with the experimental measurements, validating the microfluidic chip design. To illustrate the application in this novel microfluidic device to a real time- cell based assay, we monitored the fluorescence intensity of cancer cells stained with Mitotracker Deep Red and exposed to a chemical gradient of staurosporine. A gradient of staurosporine on cells stained with Mitotracker Deep Red (MTDR) showed a concentration-based effect on cell apoptosis across the cell culture array. Simplicity of this device in fabrication as well as in handling will be a distinct advantage in drug discovery process.

**Keywords:** Bioanalytical, Drug Discovery

**Application Code:** Bioanalytical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Microfluidics: Cells, Bacteria, Viruses

A Chiral Microchip Electrophoresis-Mass Spectrometric Platform for Studying Stereochemical Preference in Cells

In this presentation, we describe the development of a chiral microchip electrophoresis-mass spectrometric (MCE-MS) method and its application in chemical analysis of on-chip cell cultures. A novel chip design with an arm channel connecting to the middle of the MCE separation channel for delivering the chiral selector was tested for performing partial filling chiral electrophoretic separations and proven valid. 3.4-Dihydroxyphenylalanine (DOPA), glutamic acid (Glu), and serine (Ser) enantiomers, the selected neuroactive compounds, were resolved within 130 s. The analytical platform was well suited for studies of stereochemical preference in living cells because it integrated cell culture, sample injection, chiral separation, and MS detection into a single platform. Two such studies were demonstrated in this work: metabolism of DOPA and uptake of Ser by PC-12 neuronal cells. It was found that PC-12 cells metabolized L-DOPA effectively while leaving the coexisting D-DOPA intact. Ser uptake by PC-12 cells showed clearly a stereochemical preference for D-Ser. After a 2 h incubation of PC-12 cells with racemic Ser, the extracellular ratio of D-Ser over L-Ser was ca. 0.2 while the intracellular ratio about 2.

Keywords: Chiral Separations, Lab-on-a-Chip/Microfluidics, Mass Spectrometry, Metabolomics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
A bipolar electrode (BPE) is a conductive phase that in the presence of an electric field drives both oxidation and reduction reactions at opposite ends. BPEs have been used for chemical and biological sensing via electrogenerated chemiluminescence (light reporting), for the generation of chemical gradients, for electrokinetic enrichment and separation of charged species, and most recently for desalination. The advantage of BPEs is that they do not require wire leads and are therefore amenable to patterning in an array format. These advantages make BPEs attractive candidates for cell capture in an array format by dielectrophoresis. This presentation describes initial results demonstrating capture of immune cells at a single BPE by negative dielectrophoretic attraction to an ion enrichment zone generated by redox reactions at the BPE.
We introduced a novel and convenient approach to culture multiple cells in microfluidic localized arrays of chambers using one-step vacuum actuation. Eight individually addressable regions of culture chambers were integrated in one device, each only requiring one simple vacuum operation to seed cells. Using sequential injection, four cell lines were seeded in designated regions in one device with high purity (99.9%-100%) and cultured for long time. High viabilities of on-chip simultaneous culture of HuT 78, Ramos, PC-3 and C166-GFP cells for 48 h was obtained. The longest culture period for C166-GFP cells in this study was 168 h with a viability of 96%+/-10%. Also, cell proliferation in each individual side channel can be monitored. Mass transport between the main channel and side channels was achieved through diffusion and studied using fluorescein solution. The major advantage of this device is capable to perform multiple cell-based assays on one device for better comparative studies. We compared the apoptotic cell percentage of HuT 78, CCRF-CEM, PC-3 and Ramos cells after treating them with staurosporine and anti-human CD95 for 16 h. With the advantages of enhanced integration, ease of use and fabrication, and flexibility, this device will be suitable for long-term multiple cell monitoring and cell based assays.
Abstract Text

We are developing in-plane nanofluidic devices to detect virus capsids and monitor their assembly by resistive-pulse sensing. Our measurements need to resolve single particles, detect capsids and their subunits at biologically relevant concentrations, and have real-time feedback without the use of chemical labels. We have incorporated nanochannels with one or more nanopores into a microfluidic platform to improve mass transfer to the nanopores and better control the reaction times of assembly. Also, the in-plane format allows devices to be designed with nanochannels and nanopores arranged in virtually any two-dimensional pattern. Devices are fabricated by machining V-shaped microchannels into a substrate by conventional microfabrication techniques. A focused ion beam instrument is then used to mill the nanochannels into the substrate to bridge the small gaps (~50 [micro]m) between the microchannels. The dimensions of the nanopores placed along the nanochannels are typically 40-nm wide, 40-nm deep, and 750-nm long and are tailored to sense the hepatitis B virus capsids, which are 36 nm in diameter. To improve the signal-to-noise ratio of our measurements, we have designed and tested a three-channel nanofluidic circuit that has baseline subtraction integrated on-chip. On-chip baseline subtraction enables higher gain settings to be used on the current amplifier, and a signal-to-noise ratio enhancement is observed due to minimization of correlated noise.

Keywords: Bioanalytical, Lab-on-a-Chip/Microfluidics, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Microfluidic platforms are powerful analytical tools due to the reduction in reagent consumption, applicability to diverse research areas, and potential for integration of multiple components. The lab-on-chip concept of integrating multiple techniques on one device often allows improved automation and throughput compared to conventional procedures. Adapting chips for applications of cellular research is advantageous, given that the dimensions of the device and the ability to constantly perfuse cell media more closely mimic the in vivo environment. For this study, a novel PDMS chip employing multilayer fabrication techniques has been developed to monitor secretion from cultured adipocytes. Lipid metabolism is heavily controlled by adipocytes, and dysregulation of these mechanisms is implicated in obesity-related disorders like type 2 diabetes. Fatty acids and glycerol are secreted by adipocytes, and their rate of release in relation to one another could provide valuable information for understanding metabolic disease. These analytes can be detected via enzyme assays, which have been modified to create a fluorescent product. The microfluidic device integrates a cell chamber for perfusion of murine 3T3-L1 adipocytes with two reaction channel networks, wherein the cell perfusate can mix with the enzyme reagents for the separate assays. The reaction products of both assays are measured simultaneously on-chip. Through optimization of the reagent flow rates and modification of the PDMS surface, the cellular secretions can be measured within the LDR of the assays.

Keywords: Bioanalytical, Enzyme Assays, Fluorescence, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
### Abstract

A lab-on-a-chip device was created for the detection of pathogenic [i]E. coli[/i] through the incorporation of functionalized poly(vinyl alcohol) (PVA) nanofibers within a polymer-based microfluidic device. The large surface area-to-volume ratio of the nanofiber mats allowed for enhanced concentration and detection of [i]E. coli[/i] O157 cells. The incorporation of nanofibers into microfluidic channels was optimized through investigating a variety of assembly strategies, including on-chip spinning and post-spinning transfer. The procedure was simplified to require no modification of the microfluidic chips, making it a highly adaptable manufacturing procedure. Nanofiber mats that were either positively or negatively charged were spun into microfluidic channels through doping the PVA with hexadimethrine bromide (polybrene) and poly(methyl vinyl ether-alt-maleic anhydride) [Poly(MVE/MA)], respectively. [i]E. coli[/i] cells are negatively charged at pH 7, providing an initial separation scheme for removing the cells from solution. [i]E. coli[/i] cells were grown in culture, diluted to about 1 cfu/microL and pumped through the nanofiber-containing microfluidic channels. Blocking of tubing and channels was optimized to avoid loss of cells through non-specific binding. Quantitative analysis demonstrated highly effective and specific capture of the [i]E. coli[/i] cells, providing a concentration factor of 20,000. Quantitation of the cells and further specific modification of the nanofiber mats will be presented here, demonstrating the unique separation capabilities of nanofiber-modified microfluidic devices and their potential as sample preparation modules in a lab-on-a-chip device. This work was funded through the support of the Cornell Agricultural Experiment Station through federal funding project 356 and the NSF CBET-0852900.

### Keywords
- Biosensors
- Lab-on-a-Chip/Microfluidics
- Portable Instruments
- Sample Preparation

### Application Code
- Bioanalytical

### Methodology Code
- Microfluidics/Lab-on-a-Chip
Microfluidics: Cells, Bacteria, Viruses

Electrical Lysis of Adhered Cells on a Reusable Transparent 3D Printed Fluidic Device Via Removable Electrodes for In Vitro Thrombus Formation

Three dimensional (3D) printing is utilized for the rapid fabrication of a transparent, reusable fluid handling device for tissue culture, where removable electrodes with the ability to affect adhered cells are incorporated. 3D printing has previously been utilized for tissue scaffolding, but to date no reports of growing cells directly on a channel of a fluidic 3D printed device has been reported. Furthermore, this is the first account of reusable, removable electrodes being incorporated into a 3D printed device for cell lysis. With a more rapid fabrication process, reusability of the final device, and possibility of standardization via open software sharing, 3D printing offers a more attractive method to develop and utilize an in vitro thrombus mimic compared to the more widely used soft lithographic techniques utilizing polydimethylsiloxane (PDMS). The device contains a single channel, but the fabrication process easily lends itself to parallelization, 1.5 mm deep with widths varying from 5 to 1 mm. Threaded inlets allow for fittings and electrodes to be connected to the device for sample introduction and cell lysis, respectively. Endothelial cells adhere to collagen deposited onto the channel feature within the device and grow to confluency, effectively mimicking the endothelium of a blood vessel. Through manipulation of feature geometry, a low voltage power source can be used to electrically lyse cells and mimic vessel stenosis. Upon lysis, whole blood is introduced to the device using a syringe and platelet activation and adhesion to and around the injured cells, and to underlying collagen can be monitored using fluorescence microscopy.

Keywords: Bioanalytical, Electrodes, Imaging, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
It has recently been determined that dopamine release is severely attenuated in the striatum of R6/2 HD model mice. Until now, this attenuation was thought to be generalized across the entire striatum. Here, we have used fast scan cyclic voltammetry (FSCV) to measure electrically evoked dopamine (DA) release and reuptake in striatal brain slices of R6/2 mice. These measurements were taken in for distinct regions of the striatum and nucleus accumbens. Our data suggests that there are regional specific differences in the degree of dopamine release impairment that suggests that the mechanism leading to the overt phenotype in HD is not generalized. The same methodology has been used to investigate chemobrain. Recent studies comparing cognitive function before and after chemotherapy suggest that approximately 20-30% of cancer patients will exhibit lower cognitive performance after chemotherapy than would be expected. Moreover, developing an understanding of chemobrain is becoming more important as the survival rates of cancers continue to increase. FSCV has been used to measure the release and uptake of DA in chemotherapy-treated rats and has found to be attenuated. Here, we have used FSCV to address if corticosterone treatment may inhibit this DA signaling impairment.
Dopamine as a neurotransmitter is crucial to motor, motivational and reward-related function of central neural system (CNS). Dopamine is also associated with many dysfunctions in CNS, such as Parkinson’s disease (PD). We can measure dopamine in vivo in brain by fast-scan cyclic voltammetry (FSCV). In this study, we measured dopamine in a 6-OHDA model of Parkinson’s disease by infusing 2 \( \mu \)g of 6-hydroxydopamine (6-OHDA) into a male rat’s striatum. 6-OHDA causes selective degeneration of dopamine terminals and nigrostriatal dopaminergic neurons in rats, which mimics the loss of DA elements involved in PD conditions. 5-7 days after the infusion, we investigated the dopamine overflow in the rat PD model in vivo using FSCV at carbon fiber microelectrode. Carbon fiber microelectrode (200 \( \mu \)m length, 7 \( \mu \)m diameter) was placed in striatum 2.02 mm away from the center of the infusion site. Dopamine overflow was triggered by electrical stimulation (300 \( \mu \)A at 15-60 Hz) through a stimulating electrode at medial forebrain bundle. Nomifensine (20 mg/kg i.p.), a competitive dopamine uptake inhibitor, was administrated in rat. Analysis of evoked dopamine responses in both 6-OHDA-lesioned and sham-lesioned animals demonstrated that 6-OHDA-lesion has three major effects on dopamine overflow. First, 6-OHDA-lesion enhanced evoked dopamine overflow in striatum especially at low frequency stimulus (15 Hz) and short stimulus (0.2s). Second, it weakened autoinhibitory tone in striatum. Third, it increased the effect of nomifensine in striatum.
Serotonin signaling plays a key role in a great number of essential biological processes such as mood and sleep, and the serotonin transporter is a target for many drugs designed to treat psychiatric disorders, such as depression and anxiety. Drosophila is a good model system for studying the basic mechanisms underlying neurotransmission because of its simple nerve system, short life cycle and ease of genetic manipulation. Drosophila has neurotransmission pathways largely homologous to mammals. Direct measurement of endogenous serotonin changes in Drosophila has been achieved using fast-scan cyclic voltammetry with an implanted microelectrode in the central nervous system dissected from a Drosophila larva. The flies were genetically altered with channelrhodopsin-2 (ChR2), a blue light-activated ion channel, in serotonergic neurons. Previous studies have used long, continuous pulses of light to elicit serotonin release. In this study, we characterized different parameters of a pulsed optical stimulation train on serotonin release in Drosophila. With short pulsed stimulations, we were able to mimic physiologically neuronal firing patterns in Drosophila and our results were similar to electrical stimulated serotonin release in mammals. Pulsed stimulations produced less serotonin release than continuous light stimulations when maintaining the same total illumination duration. However, with the pulsed stimulations, serotonin release and uptake kinetics can be modeled using Michaelis-Menten kinetics and the effects of different stimulation parameters studied. Our results demonstrated that optical control combined with FSCV is highly flexible and effective and these stimulations will significantly strengthen the utility of Drosophila as a model system. Funded by NIH R01 MH085159.

Keywords: Electrochemistry, Microelectrode, Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Parkinson's disease (PD) is a neurodegenerative disease stemming from a selective loss of dopaminergic neurons in the brain. To date, literature reports about the effects of levodopa on oxidative stress caused by reactive oxygen species (ROS) are contradictory. The reason for these opposing results may be attributed to a lack of temporal resolution with the techniques used to measure ROS. The goal of this project is to simultaneously monitor the effects of levodopa treatment on dopamine and H2O2 dynamics in real-time using fast-scan cyclic voltammetry at Nafion-coated carbon-fiber microelectrodes. Data was collected for an hour after acute treatment with L-DOPA at a clinically relevant dose (5 mg/kg, i.p.), and following an additional injection of L-DOPA (200 mg/kg, i.p.). The administration of L-DOPA at dose of 5 mg/kg had no significant effect on either electrically evoked DA release or H2O2 fluctuations in non-lesion control animals. Conversely, L-DOPA treatment at dose of 200 mg/kg significantly increased electrically evoked DA release and decreased the frequency of H2O2 fluctuations. The data suggest that there is a dose-dependent effect of L-DOPA treatment on both DA and H2O2 dynamics. The noted decrease in H2O2 frequency suggests that L-DOPA (200 mg/kg) decreases oxidative stress in the striatum of control animals, lending support to its role as a neuroprotectant in these subjects. We will repeat this experiment in 6-hydroxydopamine lesioned rats. Importantly, this research will shed new light on the effects of L-DOPA therapy and the molecular mechanisms that underlie the emergence of motor complications associated with PD.

Abstract Text

Keywords: Electrochemistry, Microelectrode, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Scanning electrochemical microscopy-atomic force microscopy (SECM-AFM) has become an advanced hybrid technique that combines the electrochemical sensitivity of scanning electrochemical microscopy (SECM) with the superior spatial resolution of atomic force microscopy (AFM) by integration of an ultramicroelectrode into an AFM probe. In this technique, surface topography can be measured and electrochemical information collected simultaneously. A number of SECM-AFM probes, which contain gold, platinum, multi-walled carbon nanotubes, and boron-doped diamond as electrode material, have been demonstrated previously. A carbonaceous electrode material would be advantageous over noble metal materials in a cellular system due to its wide-potential scanning window, low biofouling and chemical inertness. Previously, we have demonstrated successful fabrication and characterization of carbon SECM-AFM probes from the amorphous carbon, pyrolyzed parylene C (PPC), through the use of focused ion beam milling. In this presentation, we will present our efforts to electrochemically image and probe differentiated PC12 cells that have been grown in an open-faced microfluidic channel. By stimulating PC12 cells with an elevated potassium solution, exocytosis of dopamine can be electrochemically monitored with chronoamperometry when the AFM probe is placed over the cell surface. The merits and challenges of using SECM-AFM to monitor exocytosis, as well as imaging the cells in contact-mode, will be discussed.
A Novel Kinetic Model of Voltammetric Dopamine Measurements in the CNS

Fast-scan cyclic voltammetry (FSCV) carried out in conjunction with carbon fiber microelectrodes enables detection of dopamine, a highly significant neurotransmitter, with high sensitivity and spatiotemporal resolution in the living brain. FSCV is particularly powerful when combined with electrical stimulation of the axons of dopamine neurons, because the dopamine measurements are precisely synchronized with the imposed cellular activity. This permits detailed kinetic analysis of the temporal features of the measured dopamine concentrations. Recent progress in our laboratory with this approach has produced new insights into the kinetic properties of the brain's dopamine systems. Our findings show that dopamine terminal fields are organized as patchworks of so-called fast and slow kinetic domains, each with distinct rates of dopamine release and reuptake and each with distinct responses to various dopamine-active drugs. Existing dopamine kinetic models, however, do not account for these domains nor do existing models reproduce key features of the domain-dependent responses that we routinely observe during in vivo measurements. We report here a new model that incorporates the concept of restricted diffusion and show that this new model readily predicts many features of the domain-dependent responses we have previously published. The concept of restricted diffusion in the complex environment that comprises the brain extracellular space is extensively described in the existing literature but, to our knowledge, has not been incorporated previously into kinetic models of dopamine. (This work was supported by grants from NIH: MH 075989 and NS 081744)
Serotonin is a neurotransmitter involved in vital biological processes such as appetite, sleep, learning, memory, and emotions. Accordingly, serotonin imbalances in the central nervous system are associated with neurological disorders, particularly clinical depression and alcoholism. Selective serotonin reuptake inhibitors (SSRIs) have shown success in treating depression and alcoholism (Saglam, Kayir et al. 2006). However, these medications differ in therapeutic value, and their neurochemical effects are not well understood. We have previously assessed the short-term effects (2 hours post-administration) of Escitalopram, a popular SSRI with high efficacy and high acceptability, with fast scan cyclic voltammetry (FSCV) (Wood and Hashemi 2013). FSCV is an electrochemical sensing technique with fitting temporal and chemical sensitivity to study serotonin neurotransmission (Hashemi and Wightman 2009). In this work, we study the effects of ethanol and Escitalopram over a longer temporal window. In mice; brain, serum, and SERT occupancy levels of Escitalopram have been reported to reach baseline at approximately two, four, and six hours after drug administration, respectively (Kreilgaard, Smith et al. 2008). Therefore we measured serotonin transmission for six hours after acute administration of Escitalopram (10 mg kg\(^{-1}\)) and ethanol (2 mg kg\(^{-1}\)). We would expect serotonin transmission to return to pre-drug values after 6 hours, however there is a lingering effect of delayed clearance. This effect implies dynamic and persistent neurochemical changes in response to an acute Escitalopram treatment. We study this phenomenon in terms of SERT expression in terminal brain regions throughout the 6-hr temporal window after Escitalopram administration. This study shows the complexities of the serotonergic system and its responses to Escitalopram and ethanol.
The effect of enhanced fluidity liquid (EFL) mobile phases on the performance of ion exchange separations was studied. These EFL mobile phases were prepared by dissolving liquefied gas (typically carbon dioxide) in a desired solvent. This process results in increased diffusivity and decreased viscosity of the mobile phase, while having a minimal effect on the strength and selectivity of the original solvent. As a result, enhanced fluidity liquid chromatography (EFLC) has demonstrated many advantages over traditional high performance liquid chromatography (HPLC), including lower column back pressures, decreased analysis times, and higher efficiencies. Enhanced fluidity liquid chromatography (EFLC) has also demonstrated an advantage over supercritical fluid chromatography (SFC) in that it can be used to separate polar compounds. EFLC and HPLC separations of biological compounds were carried out under strong-cation exchange (SCX) and hydrophilic interaction (HILIC) modes. Analytes included amino acids and peptides. Analysis of these compounds via EFLC methods yielded higher efficiencies and faster analysis times than HPLC methods. The effects of EFL’s on retention, selectivity, efficiency, and resolution of the separations were also examined. Funding was provided by the National Science Foundation.
Trends in stationary phase development shift to ever-smaller particles, generating larger backpressures. To accommodate for this, new U(H)PLC systems have been developed which can deal with this backpressure. As a solid support for the stationary phases in HPLC, silica poses significant advantages in terms of rigidity and multifunctionality. These are the main reasons silica is most abundantly used as a packing material in HPLC columns. However, the drawbacks of silica, the poor hydrolytical stability, are limiting the exploitation of the pH, temperature and pressure extremes of (aqueous) liquid chromatography. To accommodate for these shortcomings, a new type of hybrid silica/carbon stationary phase was developed. Additionally, the functional group is anchored to the support through a carbon bond. In this way a stationary phase is conceived that exhibits the chemical stability of carbon-based stationary phases and retains the mechanical rigidity of silica packings. The synthesis of this new type of stationary phase for HPLC as an application is described. The developed silane molecule contains an allyl function (see figure). This can be further functionalized with thiol-ene chemistry to generate any type of desired stationary phase. The column was evaluated under drastic basic and acidic conditions (from pH 1.75 to pH 12) for more than 10 000 minutes. This demonstrated the high hydrolytical stability and the preservation of the anchored retentive phase. In a second test the column was evaluated at high temperatures. This showed that the column remained stable after more than 200 injections up to 150 °C.
Separation Science: Novel Approaches to Improve Chromatographic Analysis

Evaluation and Applications of a HILIC/Cation Exchange/Anion Exchange Trimodal Column

Selectivity, mainly governed by column chemistry, is the key in HPLC separation. Although reversed-phase columns (e.g. C18) are most commonly used in pharmaceutical applications, they often fail to retain highly polar molecules (e.g. counter ions), and offer limited selectivities. Mixed-mode chromatography provides a viable solution to these challenges by using both reversed phase and ion-exchange retention mechanisms. One major advantage of this approach is that column selectivity can easily be modified by adjusting mobile phase ionic strength, pH and/or organic solvent concentration. As the 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 a given column under different conditions. Existing mixed-mode columns range from bimodal, such as reversed-phase/anion-exchange (RP/AEX) type or reversed-phase/cation-exchange (RP/CEX) type, to trimodal, such as reversed-phase/cation-exchange/anion-exchange (RP/CEX/AEX) type. While these phases provide adjustable selectivity and adequate retention for charged analytes and hydrophobic neutral analytes, they often fail to retain highly hydrophilic neutral analytes which usually retained better by HILIC interaction. This presentation will discuss column chemistry and chromatographic evaluation of a HILIC/CEX/AEX trimodal phase. Application examples in pharmaceutical, food & beverage, and chemicals will also be presented.

Keywords: Beverage, Chemical, HPLC Columns, Pharmaceutical
Application Code: General Interest
Methodology Code: Liquid Chromatography
Traditionally the carrier gas used in GC is helium. There are however increased drivers to choose a different carrier gas. This can be nitrogen or hydrogen. Especially the last years there has been a lot of discussion to use different carrier gases because of the delivery issues for helium. For many applications one can use nitrogen, but there is an impact on the chromatography. Hydrogen is also a good alternative, as it also allows much shorter run times. There are practical issues to consider in changing carrier gas. In this presentation an overview of opportunities will be presented as well as the practical concerns that has to be dealt with. Changing carrier gas is one, but we do prefer the same separations.(peak elution order). That means that changing carrier gas must also followed by a change of analytical conditions, meaning not only the pressure and split/splitless settings, but also the oven temperature program. Changing to nitrogen is possible, but it has also some serious implications that need to be considered.
UltraPerformance Convergence Chromatography (UPC2) is a separation technique that uses compressed carbon dioxide as the primary mobile phase. It takes advantage of the unique physical properties of compressed carbon dioxide (at or near supercritical state), sub-two micron particle chromatography columns and advanced chromatography system design to achieve fast and reproducible separation with high efficiencies and unique selectivity. These improvements lead to new interest in applying this technology to various industrial analytical areas, especially those areas where normal-phase liquid chromatography (NP LC) has been commonly used, such as fat-soluble vitamins (FSV), carotenoids, and lipids. Nine representative FSV and carotenoids have been successfully separated simultaneously by UPC2 within four minutes on a single C18 column. These FSV and carotenoids include vitamin A acetate and palmitate, alpha-tocopherol and its acetate, vitamin D2, vitamin K1 and K2 (MK4), beta-carotene and lycopene. The repeatability (n=6) of all the nine compounds was less than 0.25% in retention times (RT) and less than 2.6% in peak areas. The investigation of lipids separation by UPC2 showed that Bridged Ethylene Hybrid (BEHTM) silica columns provided the best separation of lipid classes among the Flouro-Phenyl, 2-EP, and BEH UPC2 columns. The lipid classes investigated include ceramides, sphingomyelin, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, lyso-phosphatidylcholine and lyso-phosphatidylethanolamine. The UPC2 has been applied to biological samples and showed successful separation of lipid classes. Separation and analysis of free fatty acids and neutral lipids was also developed. These results indicate that UPC2 is a promising chromatographic technique for FSV, carotenoids and lipids analyses.

Keywords: Lipids, Pesticides, SFC
Application Code: General Interest
Methodology Code: Other (Specify)
Ionic liquids are a class of nonmolecular ionic solvents with low melting points. These liquids are unique combination 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 our biscyanopropylpolysiloxane phases. These phases will provide unique selectivity for the evaluation of a number of petrochemical samples. 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. 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: General Interest
Methodology Code: Gas Chromatography
Dyes are added to textile products to create a desired color, and are chemically bonded with the fiber. Primary sources of dye, historically, have been plants and animals. However, we have also produced artificial dyes to obtain a more expansive color spectrum and to resist washing and wear. Different types of dyes are used for different types of fiber. For example, acrylic fibers use basic dyes, and protein fibers such as wool, are dyed with acid dyes. Cotton, however, is dyed with many dye types, including direct dyes and vat dyes. There are several important reasons for identifying dyes in fibers. Identifying dyes in very old fibers can help us learn more about ancient civilizations, and the development of textile technology. In forensic applications, dye identification can help with analysis of textiles found at crime scenes. Some synthetic dyes pose health hazards and are banned in some countries. These dyes are still widely used in other countries, so making identification may be important for regulation of imports. It also may be important in keeping industrial dyeing operations in check. Because dye molecules are non-volatile, they are often analyzed by HPLC, TLC and FTIR. GC is used for the analysis of volatile and semi-volatile components only. Analytical pyrolysis extends the use of a typical gas chromatograph to include heavier, non-volatile species, such as dyes, by breaking molecular bonds, and producing more volatile fragments of the parent molecule. Dyes have already been analyzed by reactive Py-GC/MS in ancient artwork such as paintings and textile mill wastewater. In this presentation, we will characterize different colored dyes using pyrolysis GC/MS, use these same dyes in cotton, and attempt to identify those dyes, as well as other dyes in colored textiles.
Liquid chromatography (LC) has lagged behind gas chromatography (GC) in development of hand-portable instrumentation. In this work, a new battery-operated, integrated nano-pump with stop-flow injector (24 V DC) was developed, and an on-column UV-absorption (254 nm) detector (12 V DC) was miniaturized to an acceptable weight and power usage for field operation. The pump weighs only 1.372 kg (3 lbs.) and can generate up to 110.32 MPa (16,000 psi) pressure. A major advantage of this pump is that it does not employ a splitter, since it is specifically designed for capillary column use. The volume capacity of the pump is 24 [micro]L, and sample of 60 nL can be injected. Flow rate calibration (300 nL-6.12 [micro]L per min) was performed, and an accuracy of >99.94 % was obtained. The percent injection carry-over was found to be low (RSD 0.31%). Reversed phase isocratic separations were performed using a 15.5 cm x 75 [micro]m i.d. fused silica capillary column filled with a monolith synthesized from 1,6-hexanediol dimethacrylate. Good retention time repeatability (RSD 0.09 to 0.74%) was obtained for a mixture containing an unretained marker (i.e., uracil) and a homologous series of alkyl benzenes. The short term noise of the detector is comparable to commercially available detectors (10\[sup\]5\[/sup\] AU). The detector linear range and limit of detection (LOD) were determined using sodium anthraquinone-2-sulfonate . A linear correlation coefficient (R) of 0.9996 was obtained for concentrations from 3.2 [micro]M to 6.5 mM using the new detector, and the LOD (S/N=3) was found to be 7.8 femtomoles (0.13 [micro]M).

Keywords: Capillary LC, Instrumentation, Portable Instruments, UV-VIS Absorbance/Luminescence
Application Code: Environmental
Methodology Code: Portable Instruments
Drug Discovery

Fraction Collection Using Sub 2 [micro]m UHPLC Separations: Challenges and Solutions

Liquid chromatography has benefited from the increased separation performance of sub-2-[micro]m column technology along with low dispersion instrumentation that produces sharp, narrow, and more concentrated peaks. When there is a desire to collect these small pure peaks, especially from complex mixtures, traditional fraction collection instrumentation designed for preparative HPLC conditions does not provide an adequate solution. Issues with excessive internal volumes, which can cause peak dispersion, slow collection to collection vessel movement, and collection to collection carry over are not uncommon.

To overcome these traditional limitations, an ideal solution to this problem is a purposefully built UHPLC scale fraction collector. In this poster, we will discuss some of the challenges of fraction collection at this scale along with some innovative solutions for collection of peaks that are often between 1-5 seconds wide with peak volumes that can range from 2 [micro]L to 200 [micro]L, and larger using column flow rates that can range from 100 [micro]L/min to 2 mL/min that must be accommodated. Solutions for very fast collector movement to minimize fraction loss, reduced carryover, maintenance of peak integrity between the detector and collection vessel to better manage low volume peaks will also be discussed.

Keywords: Chromatography, HPLC, Instrumentation, Liquid Chromatography

Co-Author(s) Jo-Ann Jablonski, Wendy Harrop

Date: Tuesday, March 04, 2014 - Afternoon
Time:
Room: - Exposition Floor, Back of Aisles 1
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.
Drug Discovery

Use of Entrapment to Prepare Columns Containing Alpha[sub]1[/sub]-Acid Glycoprotein for Rapid Studies of Drug-Protein Binding by High-Performance Affinity Chromatography

Many recent studies have focused on the interaction between drugs and serum proteins because of the importance of this binding in determining the transport, excretion and metabolism of numerous drugs in the body. One serum protein that is involved in these interactions is alpha[sub]1[/sub]-acid glycoprotein (AGP). This protein is a major constituent in plasma and has the ability to bind and transport numerous basic and neutral drugs in blood. High-performance affinity chromatography (HPAC) has been shown in the past to be a powerful means for studying drug-protein binding. This work examined a slurry-based entrapment method to immobilize AGP in HPAC microcolumns (10 mm × 2.1 mm i.d.) for rapid studies of drug-protein binding. The conditions needed for this entrapment process were examined and optimized, and the chromatographic behavior of the immobilized AGP was evaluated. When this type of columns was used in binding studies, the association equilibrium constant ($K_a$) measured by frontal analysis at pH 7.4 and 37°C for carbamazepine with AGP was $1.2 (± 0.6) \times 10^5$ M$^{-1}$, which was similar to a previously reported value of $1.0 (± 0.1) \times 10^5$ M$^{-1}$. Zonal elution experiments were also conducted with these columns to measure $K_a$ values for a series of drugs with AGP, including carbamazepine, disopyramide, imipramine, lidocaine and [i]S[/i]-propranolol. The experimental results again showed that the entrapped AGP had good agreement with the binding behavior that is seen for soluble AGP.

**Keywords:** Drugs, HPLC Columns, Immobilization, Protein

**Application Code:** Drug Discovery

**Methodology Code:** Liquid Chromatography
Tetracyclines are a class of medically important broad spectrum antibiotics that were discovered more than 60 years ago. Their core structure consists of four rings, of which one is usually aromatic and their biosynthesis is catalysed by complex enzymatic systems termed polyketide synthases. Massive use of this class of antibiotics led to wide-spread occurrence of resistance genes, thus there is immense need to develop new antibiotics with novel mode of action. In addition to the typical TCs, a second group so-called atypical TCs, with yet unknown mode of action, such as anhydrotetracycline, 6-thiatetracycline and chelocardin (CHD), were discovered. Considering CHD displays unusual tetracycline backbone and potent antibacterial activity with a mode of action fundamentally different from other TCs, CHD backbone can serve as suitable scaffold for biosynthetic engineering of novel antibacterials. In search for new potentially medically useful tetracyclines, fast and simple identification and determination of their structure is of great importance. MS/MS determination and quantification of these class of compounds is based on the most abundant SRM transitions, where neutral loss of amino group, water or in some cases both occurs. Up to now fragmentation of atypical tetracyclines was not studied in detail. Collision induced fragmentation of three atypical tetracyclines and two reference anhydrotetracycline epimers were studied. Fragmentation was performed using LC-MS/MS and HPLC-TOF instruments in order to obtain high resolution molecular masses for fragment ions and to compare collision induced fragmentation with in-source fragmentation. We also discuss the different fragmentation patterns of epimers.
A method was created based on ultrafast affinity extraction and high performance affinity chromatography (HPAC) for examining both the dissociation rate constants and association equilibrium constants for solution-phase drug-protein interactions. Human serum albumin (HSA), a serum protein involved in the transport of many drugs and hormones within the body, was used as a model target for these binding studies. Various drugs were used to test this method, including warfarin, tolbutamide, acetohexamide, verapamil, gliclazide and chlorpromazine. It was found that the dissociation rate constants obtained by this method were consistent with values obtained by alternative techniques. It was also found that the association equilibrium constants for these drug-protein interactions, as estimated by this chromatographic approach, were also comparable with the literature values determined by zonal elution studies or frontal analysis. In each case, these results were obtained in only a matter of several minutes. These results demonstrated that ultrafast affinity extraction and HPAC can be used as a rapid approach for the study of drug-protein interactions, providing information on both kinetic parameters and affinities of drug-protein interactions in the same experiment. The same approach could be adapted for use in future work with other drug-protein systems.
Drug Discovery

Isolation of a Bioactive Compound from Tillandsia Recurvata Plant Extract Using Supercritical Fluid Extraction and Mass Directed Preparative Liquid and Supercritical Fluid Chromatography

Natural products are a rich source of diverse chemical entities and widely used in nutraceutical, pharmaceutical and related industries. The challenges in isolating the bioactives from natural products often arise from the overwhelming complexity of the sample matrix and the wide dynamic range of the components. In this poster, we present a case study of using supercritical fluid extraction, mass directed preparative high performance liquid chromatography (HPLC), and mass directed supercritical fluid chromatography (SFC) to isolate a bioactive compound from Tillandsia recurvata plant extract. The Tillandsia recurvata plant material was first extracted by SFE. The analytical profiling of the plant SFE extract by UPLC/MS and UPC2/MS revealed a desirable orthogonality between the two chromatographic techniques in selectivity, manifested in both resolution and elution order for the peaks of interest. Leveraging the orthogonality between the two techniques, a two-step purification process using mass-directed prep LC followed by mass-directed prep SFC, was subsequently implemented, resulting in our obtaining the pristine compound in a form suitable for further bioanalysis. The purified target was also subjected to HRMS, MSE and NMR analyses, and the complete structure of the compound was successfully elucidated. The proposed workflow is generally applicable for high-efficiency natural product isolation and purification in the pharmaceutical, nutraceutical and related industries.

Co-Author(s)  Jacquelyn Runco, Jo-Ann Jablonski, John P. McCauley, Yun Alelyunas

Abstract Text

Keywords: Liquid Chromatography/Mass Spectroscopy, Natural Products, Prep Chromatography, SFC
Application Code: Drug Discovery
Methodology Code: Liquid Chromatography/Mass Spectrometry
Food safety is a known global problem, affecting millions of people that suffer from microbial contaminated foods. The antifungal capacity of essential oil (EO) and a major compound, 4 cyano-N-acetyl aniline (4CNAA) isolated from Morinda lucida root-bark oil were investigated. The M. lucida root was obtained from Forest Research of Nigeria, Ibadan, was air dried at 34°C for 96 h and hydrodistilled to obtain 1.05 % yield of the EO. TLC-DPPH was used to obtain ten different fractions. The 4CNAA purified from one of the fractions was characterized and identified by UV-visible, GC/MS and MS. Six fungi were subjected to susceptibility test against M. lucida EO, 4CAAN and fractions by plate diffusion method and their minimum inhibitory concentration (MIC) determined by micro broth dilution method. The results obtained indicated that the EO, 4CNAA and fractions were active against all fungi used (Candida albican, Candida globerate, Candida krusei, Aspergillus fumigatus, Cryptococcus neoformans, Aspergillus flavus) except the fractions in A. fumigatus. Morinda lucida root-bark EO gave the best activity at 14 mm zone of inhibition against C. neoformans. The least activity was from the fractions against A. flavus at 4 mm zone of inhibition. The M. lucida root oil MIC, 31.30 ± 0.02 μg/mL, was similar to Amphotericin B (standard drug) in C. krusei. The antifungal potential of the M. lucida root-bark essential oil is noteworthy as an active agent against skin diseases and as a natural food preservative.
Drug Discovery

Analysis of Drugs: Single Fast Approach for the Determination of Most Common Drugs and their Metabolites Using GC-TOF-MS

The Drug abuse is the use of illegal substance or the misuse of prescription or over-the-counter drugs for at least a year with negative consequences. Throughout the world, illicit drug use appears to be generally stable, though it continues to be rising in several developing countries. Drug abusers number is about 27 million, which is 0.6 per cent of the world adult population, but about 230 million people, or 5 per cent of the world’s adult population, are estimated to have used an illicit drug at least once in 2010 (World Drug Report 2012, UNODC).

As consequence, drug screening technology has improved greatly, in order to provide better and faster quantification in biological matrices.

The aim of this work was to develop a complete analytical method using a fast Gas Chromatograph coupled with a Mass Spectrometer with Time Of Flight technology (TOF MS). This system allows a unique fast analysis (less then 8 minutes) of the most common and used drugs (Cannabis, opioids, opiates and Cocaine), their metabolites and their adulterants from biological and autopsy samples with outstanding linearity, sensibility and repeatability. With TOF MS, thanks to the full acquisition range, it’s possible to identify both known and unknown compounds in complex matrix (serum and urine) at very low concentrations, increasing the information about the real samples.

Keywords: Clinical/Toxicology, Drugs, Forensic Chemistry, GC-MS

Application Code: Drug Discovery

Methodology Code: Gas Chromatography/Mass Spectrometry
Azadirachta indica (Neem tree) is used in traditional medicine in many West Africa countries for the treatment of human diseases. The seeds were dried at 28 °C for 8 days and the neem crude oil (NCO) extracted by mechanical press. Two hundred mL of the NCO was refined with 10 % activated charcoal at 65 °C. The oil after cooling was decanted, centrifuged at 500 rpm for 10 min, filtered and further treated with 5.4 % clay. The compositions of NCO and neem refined oil (NRO) were obtained by high resolution GC/MS method. The GC/MS analysis shows that the NCO and NRO contained 9 and 13 constituents respectively. Except hexanedioic acid and aldehyde in the NRO, the two oils have common constituents. The dominant constituents in the NCO and NRO were 6-octadecenoic acid (30.42 %), hexadecanoic acid (21.51 %), methyl octadecanoate (16.88 %) and 6-octadecenoic acid (31.22 %), stearic acid (17.26 %) respectively. Physico-chemical analyses of the extracted NCO shows it has a brownish colour, smell similar to garlic, the moisture content, refractive index and pH are 0.64 %, 1.41 and 5.24 respectively. The NRO was colourless, de-bittered, the moisture content, refractive index and pH are 0.56 %, 1.24 and 4.64 respectively. The antimicrobial results of the soap produced from the oils, indicates that NCO has more antimicrobial activities than the NRO against some commonly encountered bacteria strains and fungi species such as Escherichia coli, Staphlococcus aureus, Pseudomonas aeruginosa, Microspora audinii and Aspergillus niger.

Keywords: GC-MS, Identification, Medical, Natural Products
Application Code: Drug Discovery
Methodology Code: Gas Chromatography/Mass Spectrometry
4-(1H-naphtho[1,8-de][1,2,3]triazin-1-ylsulfonyl)-N-arylidene aniline (2a-e) was synthesised by a facile condensation of aromatic aldehydes with 4-(1H-naphtho[1,8-de][1,2,3]triazin-1-ylsulfonyl)aniline (1) in good yield. Cyclo condensation of compounds (2a-e) with Succinic anhydride yields 1-(4-(1H-naphtho[1,8-de][1,2,3]triazin-1-ylsulfonyl)phenyl)-5-oxo-2-arylpyrrolidine-3-carboxylic acid (3a-e). The structures of these compounds were established on the basis of analytical and spectral data. All the newly synthesized compounds were evaluated for their antibacterial and antifungal activities.
1-(3,5-dibromo-2,4-dihydroxyphenyl)ethanone (HBBA) has been prepared by the coupling of benzyl bromide and 3,5-dibromo resacetophenone. Some novel chalcones have been synthesized by its condensation with various aromatic aldehydes. The newly synthesized compounds were characterized by elemental analysis, IR and NMR spectral analysis. The antimicrobial activity of prepared compounds have also been checked.
Drug Discovery

Application of Soya Based Nanosponges for Monitoring Thermal Degradation Products of Epoxy Insulators in Electrical Transformers

Epoxy insulators used in electrical equipment such as dry type transformers are amorphous polymers synthesized from epichlorohydrin - bisphenol oligomers cross-linked using amines, anhydrides or carboxylic acids. During transformer operation coils generate heat. Adverse conditions such as high loads and/or with high ambient temperature can lead to excessive heat buildup which in turn can cause degradation of insulator and the failure of the transformer. A study was initiated to monitor evolution of degradation products as a function of temperature. The aim of the study was to develop an understanding the degradation pathway through quantification of degradation products. Thermal decomposition of the material was examined with two different approaches. In the first approach temperature of the epoxy insulator sample was raised in a step vise manner and degradation products were trapped on soya based nanosponges. Similarly, nanosponge cartridge were used for trapping epoxy insulation degradation product in vicinity of operating dry type transformers. Degradation products from laboratory simulation experiments and operating transformers in the field were determined with gas chromatography – mass spectrometry (GC-MS). The nanosponges proved to be effective adsorbent for degradation products and permitted their quantification. The major degradation product was bisphenol A, this compound accounts for nearly 40% of the total semivolatiles produced during thermal decomposition at high temperatures other thermal degaration products including o,m and p hydroxytoluene and iso-propyl phenol were also observed.

Keywords: Absorption, Capillary GC, GC-MS, Polymers & Plastics
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Consumer products are often stored in large warehouses and transported in trucks with a variety of other products. As the temperature of these facilities rise, the compatibility of the products can become an issue. If a highly odorous product is stored too closely to, or shipped with, an odor absorbent product, odor and taste issues can result. In this work, solid phase microextraction (SPME) was used to sample the air around odorous consumer products at various distances. The samples were analyzed via gas chromatography (GC) coupled to mass spectrometry (MS) and olfactory detection, run in parallel. This allowed for odors to be recorded even when the concentration was too low for the MS detection, but still significantly high enough for human detection. As the distance from the odorous product increased, the odorants detected decreased, until a distance was established where the odor was no longer absorbed by the SPME fiber. At this distance then, it could be assumed that odor absorbing products would also no longer be affected by the odorants. The results of this work could be used to establish appropriate storage distances in warehouses and the compatibility of products that may be shipped together. Additionally, the use of SPME fibers allows for some selectivity of the components being sampled through the choice of fiber coating, therefore further tuning of the method could be performed if needed.
Heavy metal ions such as mercury, and lead are readily complexed by bis(1-pyrenyl)azine. The later is prepared in a one-step reaction from 1-pyrenecarboxaldehyde and hydrazine hydrate. It has been previously reported that this receptor has unique optical and fluorescent sensing abilities toward Hg$^{2+}$ and Cu$^{2+}$ ions. Our efforts have been focused on the separation of a series of heavy metals using a radial TLC device with fluorescence detection. The technique and separation capability of bis(1-pyrenyl)azine be presented.

The Finnish Defence Forces has destroyed obsolete and spoiled ammunition in open surface mass detonations. The destruction camp is organized every year and lasts five weeks. One charge is detonated every day. The material to be destroyed consists of out-of-date and spoiled ammunition. Gross weight of an average charge is 200 tons consisting of 20 tons of military explosives and 3 to 5 tons of emulsion explosives as a booster.

The Finnish Defence Forces Technical Research Centre (PVTT) has studied the environmental effects of the mass detonations since they began in 1988. The environmental effects of the explosions can be divided into three classes; 1) gaseous emissions, 2) explosive residues and their conversion products and 3) metal emissions.

PVTT has developed a method for determination heavy metal deposit range of open surface mass detonations. On the surface of the spruce needle, Picea abies (L.) Karst, there is a very thin layer of wax, which works as an effective trap for the airborne impurities. The spruce needle samples were collected as a function of distance measured from the detonation site down to 10 km. The locations of the sites were determined by the GPS system. The wax layers of the needle samples were extracted by chloroform and their metal content were analyzed by the ICP-MS-instrument. By plotting the metal concentrations of the wax (\text{g/g}) as a function of distance measured from the detonation site, one gets a curve by which the heavy metal deposit range can be estimated. Spruce needles, Picea abies (L.) Karst, can also be used as an effective bio indicator to estimate the heavy metal deposit range from the spot like metal sources such as open surface mass detonations, burning sites of propellants, metal factories, foundries, open pits or waste burning plants.
### Session Title
Environmental Analysis of Toxic and Persistent Compounds

### Abstract Title
In-Situ Electrochemistry of Extreme Environments on Earth

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**Co-Author(s)**

**Abstract Text**
The application of in-situ electrochemistry to extreme environments on Earth will be presented. Hydrothermal vent environments on the ocean floor using both the Alvin submarine and the Jason remote operated vehicle will be shown. In addition the analysis of the most naturally polluted river on Earth, the Rio Tinto, Spain, and the profiling of Antarctic lake basins using cyclic and squarewave voltammetric techniques coupled with unique new micro-electrodes will be highlighted.

**Keywords:** Electrochemistry, Environmental, Geochemistry, Voltammetry

**Application Code:** Environmental

**Methodology Code:** Electrochemistry
## Session Title
Environmental Analysis of Toxic and Persistent Compounds

## Abstract Title
**GC-PID for In-Situ Soil Investigation**

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### Abstract Text
For a long time, low environmental sustainability standards led to soil contamination in industrial areas. Organic solvents such as organochlorides are typical agents that can occur. These harmful substances persist for many years. For redevelopment of these brownfield lands sites, ground surveys have to be undertaken. A quick response system is needed to detect hot spots of contamination on-site.

A GC-PID was developed to analyze the contaminated soil in the field. The in-situ instrument attains different horizons of contamination by a hollow stem auger. The inner measurement unit is sheltered in a ruggedized stainless steel cylinder. Ground water can be detected by a resistive humidity sensor. An additional camera at the stems flank allows an optical impression of the surrounding soil. At certain soil layers, an inlet at the tip of the auger is opened so that a gas sample can enter the instrumentation. The measurement device is composed of a 10.6 eV photo ionization detector and a temperature controlled short gas chromatographic multi capillary. The GC injector has a special valveless design for this application. The carrier gas for the chromatography is generated by a molecular sieve of activated carbon. The system setup and first field tests will be presented.

### Keywords:
- Contamination
- Environmental/Soils
- Gas Chromatography
- Portable Instruments

### Application Code:
Environment

### Methodology Code:
Gas Chromatography
Cocaine is one of the most widely used illicit substances around the world. Benzoylecgonine is a main metabolite or decomposed product of cocaine. Several recent studies have shown that the abuse of cocaine contaminates paper currencies and sewage water [Zuo, et al. 2013, In: Y. Zuo, Eds., High-Performance Liquid Chromatography (HPLC): Principles, Procedures and Practices. Nova Science Publishers, Inc.]. The extent of these contaminations provides valuable epidemiological information about the drug abuse. In this study, a GC-MS method was developed for the simultaneous determination of cocaine and benzoylecgonine in paper currencies and sewage water. The procedure involved the extraction of analytes using water and solid-phase extraction, followed by the derivatization of benzoylecgonine with BSTFA (N,O-bis(trimethylsilyl) trifluoroacetamide) + TMCS (trimethylchlorosilane) reagent. The trimethylsilyl derivative formed and cocaine were separated and quantitated using GC-MS.
Environmental Analysis of Toxic and Persistent Compounds

Single Column Analysis of PBDEs, Including BDE 209

Polybrominated Diphenyl Ethers (PBDE’s) are aromatic and non-polar compounds that were used as flame retardants. After extensive usage, it was determined that these compounds are toxic and have been restricted or banned in many areas, including under the Stockholm Convention.

PBDE’s consist of 209 individual congener conformations which vary in toxicity. It is therefore important to measure and quantify the most toxic of these individual congeners separately. To achieve the lowest levels of detection and highest degrees of confidence, high resolution gas chromatography with high resolution mass spectrometry (HRGC/HRMS) is used. Even using this advanced instrumentation, accurate separation of all congeners is difficult and requires long run times to provide resolution. In addition, not all congeners are stable and may degrade if activity exists in the system. One example is the most substituted and latest eluting congener BDE-209, which often requires a separate analysis using a shorter column to reduce activity and provide sufficient response.

This work utilizes a new method and technology that allows for fast quantitation of toxic congeners with short run times, and includes the quantitation of congener BDE-209 in the same analytical run. This eliminates the need for an extra instrument using an alternative column dimension to quantitate the necessary congeners. Comparison of existing methods and the proposed method are included highlighting improved sensitivity and shorter run times.

Keywords: Environmental Analysis, Gas Chromatography/Mass Spectrometry
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Contamination of food products with pesticides is a growing concern because of recognized adverse health effects, increasing world-wide usage of pesticides, and increasing imports of raw foodstuffs from foreign sources. Gas chromatography mass spectrometry (GC/MS) has been used extensively to quantify trace level pesticides in food matrices; the most significant challenges have been matrix interference and achievement of meaningful health-based detection limits for the compounds of interest. The QuEChERS (Quick Easy Cheap Effective Rugged and Safe) sample preparation method has helped to overcome some of the problems of matrix interference, and commercialization of QuEChERS kits has promoted widespread screening of foodstuffs for trace pesticides. Triple quadrupole GC/MS/MS operating in the Multiple Reaction Monitoring (MRM) mode has emerged as the technique of choice for analysis of trace level contaminants in complex matrices.

This poster presents instrument configuration, operating parameters, and analytical results for analysis of trace levels of 37 pesticides of various chemical classes in a QuEChERS extract of baby food using the MRM mode on a triple quadrupole GC/MS/MS. Results were evaluated for calibration linearity, analytical precision, and accuracy. The effect of MS resolution on compound selectivity in a complex food matrix is also discussed.

**Keywords:** Food Science, Gas Chromatography/Mass Spectrometry, Pesticides, Tandem Mass Spec

**Application Code:** Environmental

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Environmental Analysis of Toxic and Persistent Compounds

Determination of Paraquat and Diquat in Environmental Samples Using a Sub-2-µm, Solid-core Particle HILIC Column

The doubly charged quaternary ammonium compounds paraquat and diquat (quats) are common herbicides used worldwide. These compounds are commonly determined in environmental water samples by ion-pairing reversed-phase liquid chromatography with UV detection. However, the ion-pairing reagents used are not usually suitable for LC-MS analysis. In recent years, HILIC-based LC methods have been demonstrated that require no ion-pairing, and thus are more suitable for LC-MS analysis. However, the HILIC approach has not been widely adopted for UV detection because baseline separation of paraquat and diquat is difficult. In this poster, we demonstrate the unique selectivity and efficiency of a sub-2-µm, solid-core particle HILIC column for baseline separation of diquat and paraquat. This column can be used for LC with either UV or mass spectral detection. In this study, both means of detection were used independently or in series. With the mass spectrometer, detection limits well below 100 ng/L (ppt) were easily achieved for tap-water samples. Using UV detection, detection limits below 500 ng/L were achieved. The samples (10 mL) were processed using a mixed mode weak cation exchange SPE cartridge prior to LC analysis.

Keywords: Environmental/Water, Liquid Chromatography/Mass Spectroscopy, Pesticides, Solid Phase Extraction

Application Code: Environmental

Methodology Code: Liquid Chromatography/Mass Spectrometry
One of the difficulties associated with the determination of polycyclic aromatic hydrocarbons (PAHs) in environmental and food samples is the complexity of PAH mixtures in these samples. Individual PAHs differ significantly in their toxicological properties. The USA EPA and European legislation have established a list of polycyclic aromatic hydrocarbons (PAHs) to be monitored in environmental and food samples. The list consists of 16 priority PAH of USEPA method 610 and 15+1 PAHs of the European protocol for food monitoring. The separation and analysis of isomeric PAH in this list is challenging. Especially, the separation of benzo(j) fluoranthene from other isomeric benzo (b) and (k) fluoranthenes, and triphenylene from benz(a) anthracene and chrysene is essential to meet the regulatory requirements. Currently, the investigated NSP-PAH columns of various lengths and internal diameters provide the separation of 32 PAHs, except triphenylene. The novel NSP-EUPAH column provides the required separation of 32 PAH, including triphenylene. The results of the separation of all 32 PAHs including triphenylene on short NSP-EUPAH columns of lengths 8 m, 10 m and 12 m are illustrated. GC-MS/EI/SIM mode was used to determine the limit of detection for NSP-EUPAH columns.
The two primary valence states of chromium are trivalent (Cr III) and hexavalent (Cr VI). Chromium III and Cr VI exposure occurs from both anthropogenic and natural sources. Hexavalent chromium is typically a toxin resulting from anthropogenic activity and is categorized as a known carcinogen. Individuals may be exposed to hexavalent chromium by breathing contaminated air, ingesting or inhaling contaminated soils or by drinking contaminated water. It is important in the environment to determine the anthropogenic generation of hexavalent chromium to prevent many health hazards. There is a need to develop soil reference materials for hexavalent chromium due to lack of solid standards readily available.

High-Purity Standards has developed reference materials within the scope of ISO 17025 for hexavalent chromium in soil. The reference materials were spiked and homogenized with varying levels of hexavalent chromium salts to represent soil samples that could be found in a contaminated environment. Analytical digestion methods were utilized to efficiently dissolve the soil sample.

Trivalent and hexavalent chromium were separated and analyzed by HPLC/ICP-MS. A chromium speciation kit from Applied Isotope Technologies, Inc. was utilized to determine Cr+3/Cr+6 interconversion. Precision obtained was $\pm 1\%$ RSD for 3 replicates. A bias of $\sim 1\%$ was determined for samples. The analytical limits of detection were calculated to be $0.05 \mu g/L$, which is sufficient in determining toxic levels of hexavalent chromium contamination in soil.
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”. The new dry air sampling device allows sensitive and reliable detection coupled with convenient and safe use. The impregnated media in the sampler form stable isocyanate derivatives and permit the shipping of the sampler to the testing laboratory without the need for field desorption using hazardous solvents. 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 new sampling device will derivatize both the isocyanate monomers and oligomers. The aromatic monomers include toluene diisocyanates-2,4-TDI and 2,6-TDI; 4,4'-diisocyanato-diphenylmethane (MDI); phenyl isocyanate (PhI). Examples of aliphatic monomers include isophorone diisocyanate (IPDI), 1,6-hexamethylene diisocyanate (HDI); and ethyl isocyanate (EIC). HDI polymeric species include HDI-Isocyanurate and HDI-Biuret, and IPDI polymers is IPDI-isocyanurate. We performed the field testing of the dry sampler during the simulated spray paint coating. We have quantitatively analyzed the air for monomeric isocyanates. The results of the study will be reported.
To ensure people safety and a good repeatability of industrial process, the analysis of ambient air is crucial. Especially, electronic boards are produced in clean air room by complex lithographic process using very reactive chemicals. The nature and concentration of volatile compounds can be different depending on the chemical process and can also vary rapidly. There is a need to analyze precisely and continuously gas process in air with an instrument designed for industrial use. Since 1986, our company is a worldwide recognised expert in gas analysis, renowned and certified for its precise analysis in ambient air monitoring and natural gas. In industry, Chromatotec’s systems prove their value in online monitoring, quality control and environmental protection. They make substantial contributions to process control, to the improvement of product quality, and to the enhancement of system safety as well as to environmental protection. Chromatotec has developed a turnkey solution which allows the quantification and identification of compounds at ppt, ppb, ppm and % levels. The airmoTWA is a new industry standard for online and continuous TRAP-GC-MS-FID. It encompasses a specific trap to concentrate the sample, a column for separation of chemicals and two detectors: a new micro flame ionization detector (FID) and a mass spectrometer for quantification and identification respectively. The airmoTWA is simple to use and incredibly sensitive and delivers robust and reliable performance. Particularly, the instrument can monitor high and low concentration for a large number of molecules. The instrument is measuring and recording concentrations of chemicals and can have alarm systems which can be set to inform on important changes of the surrounding atmosphere.

Abstract Text

Keywords: Gas Chromatography, GC-MS, Trace Analysis, Ultratrace Analysis
Application Code: Process Analytical Chemistry
Methodology Code: Gas Chromatography/Mass Spectrometry
Environmental: Air Analysis

Characterization of Low and Non-Volatile Organics in Particulate Matter Using Thermal Extraction Followed by Pyrolysis with Gas Chromatography Mass Spectrometry

Organic matter (OM) represents 10–70% of air particulate matter (PM), with approximately 80% of OM currently being uncharacterized. This unresolved fraction has been suggested to be composed of either a mixture of low molecular weight (LMW) species or non-volatile high molecular weight (HMW) compounds that are not congenial with the commonly deployed gas chromatographic mass spectrometric (GC/MS) analysis. In this work we have developed a method utilizing sequential thermal extraction/pyrolysis with gas chromatography mass spectrometry (TE/Py-GC/MS) for detailed characterization of carbonaceous PM. This method enabled the separation of LMW compounds recovered using TE, followed by pyrolysis, which provided a characterization of the HMW fraction. However, in order to effectively employ this method, understanding of the matrix-analyte interactions is essential. Initial TE/Py-GC/MS experiments showed a significant contribution of non-covalent interactions affecting the desorption of LMW species (compounds previously detected in real-world polar PM samples) adsorbed on either graphite or silica particles. The most pronounced effect was observed for 3- and 4-ring polycyclic aromatic hydrocarbons (PAHs). To alleviate the ambiguity arising from the observed strong matrix-analyte interactions in PM samples, solvent extraction (SE) steps (with water followed by a series of organic solvents) was implemented, and then the TE/Py-GC/MS analysis was performed on both the solvent extracts and the remaining residual PM. The developed SE/TE/Py-GC/MS method was validated on the model wood smoke PM as well as PM collected on filters during a 16-week air sampling campaign in Grand Forks, ND.

Keywords: Aerosols/Particulates, GC-MS, Pyrolysis, Sample Introduction
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
During the summer months of 2002, 2003, and 2004 air pollution studies were completed in Chicago, Illinois. Trace gases and meteorology were measured concurrently while two aerosol samples were collected each day. Canonical correlation analysis (CCA) and principal component analysis (PCA) were applied to ammonium, calcium, nitrate, sulfate, and oxalate concentrations along with temperature, wind speed, wind direction, and humidity data. The purpose of statistical analysis was to determine the extent of relationships between meteorology and pollutant concentrations as well as reduce data dimensions to identify patterns or outliers observations.

As a result of CCA, three statistically significant canonical functions were derived. Canonical weights revealed both ozone and oxalate had a linear correlation with temperature. Humidity and wind speed were shown to influence local ammonium concentrations as well as trace gases. Nitrogen oxides and residuals of ozone both had an inverse relationship with wind speed. Similar information was uncovered in canonical loadings, in addition to correlations partialed out in canonical weights due to collinearity. In PCA, dimensionality was reduced from 11 to 4, with derived linear combinations accounting for 77.0% of original data variance. Principal components suggested production/transport of secondary aerosols to the Chicago region. Ozone, oxalate, sulfate, nitrate, and ammonium were positively associated with temperature. Wind speed’s influence on pollutants as well as photochemistry of ozone were also represented in principal components. Relationships between only meteorological parameters were derived as well in one linear combination. Several clusters as well as outliers corresponding to high pollution days were identified in CCA and PCA score plots. Overall, by reducing data dimensions and determining variable relationships, regional pollution variability can better understood and data is more manageable.
In the frame of the "Austrian Space Applications Programme" (FFG-ALR, BMVIT) IONICON Analytik, in tight collaboration with the University of Innsbruck, designed and constructed a compact and rugged Proton-Transfer-Reaction Time-Of-Flight Mass Spectrometry (PTR-TOFMS) instrument for atmospheric trace gas measurements. The instrument (see photograph below) was custom-built for airborne deployment and it includes shock and vibration protection of the orthogonal acceleration reflectron TOFMS and an AC/DC/AC universal power supply for stable power generation.

A drawback of quadrupole-MS based airborne PTR-MS instruments is their limited specificity at unit mass resolving power and their low scanning speed. Here we present performance evaluation data proving that the PTR-TOFMS overcomes these two problems. A mass resolving power $m/\Delta m$ of up to 1500 was sufficient to separate isobaric compounds (e.g. ethanol and formic acid), and full mass spectra were typically recorded with one second signal integration. Utilizing certified gas standards we found a sensitivity of 130 cps/ppbv (for xylene) which leads to a limit of detection of 50 pptv for one second integration time.

We will present exemplary data from first airborne deployments of the new PTR-TOFMS instrument in the frame of NASA’s DISCOVER-AQ (Deriving Information on Surface Conditions from Column and Vertically Resolved Observations Relevant to Air Quality) mission. PTR-TOFMS measurements onboard the NASA P-3B Airborne Laboratory provided important data on pollution sources and photochemical processes in selected areas of the U.S. which are affected by poor air quality.

**Keywords:** Chemical Ionization MS, Environmental Analysis, Time of Flight MS, Ultratrace Analysis

**Application Code:** Environmental

**Methodology Code:** Mass Spectrometry
A number of volatile organic compounds (VOCs) – and sulfur compounds in particular – are a challenge for the analyst because they can decompose at the high temperatures used by GC–MS analytical equipment. In addition, some of them, such as hydrogen sulfide and methanethiol, are very volatile, making them difficult to sample and analyse quantitatively. At the same time, sulfur compounds are associated with unpleasant odours even at very low concentrations, which can be a cause of negative public image. Releases from factories, sewage treatment plants and landfill sites can all contain odorous sulfur compounds, and so there is a need for frequent (or continuous) monitoring of these sites, preferably with rapid response in order to identify and eliminate any undesirable odours.

This poster will present a solution to the challenge presented by rapid and reliable detection of sulfur compounds, employing thermal desorption as a preconcentration technique for GC–MS analysis. The thermal desorption systems described use a completely passivated flow path for compatibility with even the most labile of sulfur species, while the large sample volumes that can be accommodated allow maximum sensitivity. Examples will be shown of how this technology can be used for continuous near-real-time monitoring using a dual-trap system, and with canisters for optimum off-line analysis of the most volatile species.

**Keywords:** Gas Chromatography/Mass Spectrometry, Sulfur, Thermal Desorption, Volatile Organic Compounds

**Application Code:** Environmental

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Recoveries of 65 VOCs Over a 30 Day Period in Dry and Humid Conditions in Two Silicon-Lined Canister Types

The recoveries of 65 volatile organic compounds (VOCs) typically analyzed as part of the U.S. Environmental Protection Agency’s (EPA’s) Compendium Method TO-15, were evaluated for two commercially available silicon-lined canisters. The VOC recoveries were determined both in the presence of dry air (0% relative humidity (RH)) and humid air (93% RH), over a 30 day storage period. The results indicate that for the majority of the 65 VOCs, there was no statistically significant difference in recoveries and stabilities between the two canister types. However, the results indicate that recoveries and stabilities were different between dry and humid conditions. The detailed experimental design, analytical techniques, results, and implications will be discussed in this presentation.

Keywords: Air, Environmental Analysis, Environmental/Air, GC-MS
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Environmental: Air Analysis

Detection of Combustion Effluents in Atmospheric Particulate Matter 2.5 (PM2.5)

Air pollution by particulate matter 2.5 (PM\(_{2.5}\)) is a common concern in East Asia. In 2009, environmental quality standard for PM\(_{2.5}\) was set in Japan and influence of long-range transport was suggested in early spring of 2013 due to occurrence of severe air pollution in eastern part of China. This study then aims to understand the actual situation of PM\(_{2.5}\) and its component for further control in East Asia. PM\(_{2.5}\) samples were collected on the quartz fiber filter by PM\(_{2.5}\) sampler at a flow rate of 16.7 L/min for 7 days in rural area in Shimane, west coast of Japan, from February to March, 2013 and urban area in Kanagawa from April to May, 2013. After measurement of PM\(_{2.5}\) mass concentration, water soluble components were determined by ion chromatography. Observation of individual fire particles and elemental analysis with mapping technology were also conduct for selected samples by electron probe micro analyzer (EPMA). PM\(_{2.5}\) ranged from 7.7 µg/\( \text{m}^3 \) to 33.3 µg/\( \text{m}^3 \) in Shimane and from 5.3 µg/\( \text{m}^3 \) to 15.4 µg/\( \text{m}^3 \) in Kanagawa. A typical spherically carbon particles emitted as coal fly ash was found in Shimane samples by EPMA. Sulfate was the most abundant in water extracts of PM\(_{2.5}\) in both cities. Elemental mapping successfully distinguished the individual sulfate particles originated from sea spray or others, comparing with the distribution of sodium, calcium and potassium. These results showed that sulfate was a key factor for pollution control of PM\(_{2.5}\).

Keywords: Aerosols/Particulates, Analysis, Environmental Analysis, Environmental/Air
Application Code: Environmental
Methodology Code: Chemical Methods
Direct Mercury Analysis is a technique that is ubiquitous in analytical labs within the environmental industry, petrochemical industry and specialty chemicals industry for analyzing mercury in solid and liquid samples. However, these labs are frequently required to test for mercury in gaseous samples in addition to utilizing specialty instruments. In addition to continuous emission testing and long term testing for relative accuracy test audits (RATA) regulations in coal production and processing, several industries have driven a need for mercury gas testing and analysis. Most conventional systems do not offer the flexibility of analyzing for these samples, which has posed a problem for direct analysis.

To address the need for a highly flexible system, Milestone Inc. has developed sorbent tubes and a field mass controller to trap mercury gases and subsequently analyzed on our standard Dual Cell (DL – 0.003 ng) or Tri Cell (DL – 0.001 ng) DMA-80 Direct Mercury Analysis Systems, offering a user the capability to directly analyze gases along with solid and liquid matrices without sample preparation. The sorbent tubes contain a special mercury adsorbing material, which traps the mercury from the gases as they flow through the tubes. These mercury containing sorbent tubes can be directly placed on the auto-sampler tray and be directly analyzed along with the regular solid or liquid samples. We will present data on the flexibility of analyzing gas samples along with routine operation of mixed samples.
Environmental: Air Analysis

Method Development for Determination of Trace Concentrations of Aldehydes and Carboxylic Acids in Particulate Matter

Carboxylic acids and aldehydes are present in ambient particulate matter originating from both primary and secondary organic emissions. Within secondary atmospheric reactions aldehydes readily oxidize to acids as well as participate in reactions with acids leading to formation of polymers. The activity of these reactive species in atmospheric processes demonstrates a need for their accurate determination. We have developed a PFBHA/BSTFA derivatization method for gas chromatographic mass spectrometric analysis enabling sequential derivatization of carbonyls and acids in atmospheric samples. The advantage of the proposed protocol is the initial methylation of carboxylic acids (in contrast to trimethylsilylation) and thus easier MS interpretation without interference from numerous hydroxyl groups, while at the same time PFBHA stabilizes reactive aldehydes. The sequential BSTFA completes derivatization of hydroxyl groups. Two solvent systems were tested (acetonitrile/dichloromethane/methanol, 1:8.5:0.5 v/v/v; and methanol only), where the first solvent system containing effectively derivatized all acid forming methyl esters and trimethylsilyl (TMS) derivatives, and the second system containing solely methanol produced only methyl esters for a broad range of acids, with exception of aromatic acids, nevertheless we were able to address their quantification using the TMS derivatives. It was also observed that overnight (18 hours) sonication is crucial for the complete derivatization.

Funding was provided by EPSCoR Sunrise and University of North Dakota (Grant 437002320UND0014548).

Abstract Text

Carboxylic acids and aldehydes are present in ambient particulate matter originating from both primary and secondary organic emissions. Within secondary atmospheric reactions aldehydes readily oxidize to acids as well as participate in reactions with acids leading to formation of polymers. The activity of these reactive species in atmospheric processes demonstrates a need for their accurate determination. We have developed a PFBHA/BSTFA derivatization method for gas chromatographic mass spectrometric analysis enabling sequential derivatization of carbonyls and acids in atmospheric samples. The advantage of the proposed protocol is the initial methylation of carboxylic acids (in contrast to trimethylsilylation) and thus easier MS interpretation without interference from numerous hydroxyl groups, while at the same time PFBHA stabilizes reactive aldehydes. The sequential BSTFA completes derivatization of hydroxyl groups. Two solvent systems were tested (acetonitrile/dichloromethane/methanol, 1:8.5:0.5 v/v/v; and methanol only), where the first solvent system containing effectively derivatized all acid forming methyl esters and trimethylsilyl (TMS) derivatives, and the second system containing solely methanol produced only methyl esters for a broad range of acids, with exception of aromatic acids, nevertheless we were able to address their quantification using the TMS derivatives. It was also observed that overnight (18 hours) sonication is crucial for the complete derivatization.

Funding was provided by EPSCoR Sunrise and University of North Dakota (Grant 437002320UND0014548).

Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Biogas processing is a rapidly growing market, and with initiatives such as the Kyoto Protocol ‘Clean Development Mechanisms’, there will be an increased need for power generation using biogas from landfill sites, wastewater treatment plants and anaerobic digesters. This has prompted interest in biogas in regions such as Europe, where the regulatory environment is favourable to this technology. However, biogas can contain a high level of siloxanes, which are a common addition to consumer products and do not decompose in the activated sludge process. The situation is exacerbated by addition of anti-foaming agents containing silicones, which biodegrade to form siloxanes.

During combustion of biogas, siloxanes are usually converted into silicon dioxide particles, which can cause significant internal damage to turbines and other motors. As a result, monitoring the levels of siloxanes in biogas has become an important topic. However, current GC–MS analytical methodology for siloxanes using canister sampling has certain fundamental flaws, and this poster will describe the advantages of an alternative methodology using thermal desorption sorbent tubes.

**Keywords:** Environmental/Waste/Sludge, Gas Chromatography/Mass Spectrometry, Thermal Desorption, Volatile

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

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Environmental: Water

Potential Contamination of Fluoroquinolones in Water-Bodies During the Production of Broiler Chicken

Leila A. Figueiredo
Universidade de Sao Paulo


Abstract Text

Brazil is the largest exporter of chicken meat and its production employs many veterinary drugs, including antimicrobials from the group of fluoroquinolones. There are still questions about what environmental impacts the constant use of antibiotics can bring. The aim of this study was to evaluate if antimicrobials applied during the process of broiler can indirectly reach water-bodies near the barracks and cause contamination. The methodology was to analyze water samples by on-line SPE-LC-MS/MS (on-line solid-phase-extraction liquid-chromatography-tandem mass spectrometry) to quantify three molecules currently used: norfloxacin (NOR), enrofloxacin (ENR) and ciprofloxacin (CIP). Samples were collected in April/2013 from water-bodies near the barracks, in seven farms from Piracicaba (SP/Brazil). Chromatographic conditions: column type: C18, 3.0 x 100 mm, 3.5 µm; binary pump mobile phase: A - 80% H2O + 0.1% CH2O2 and B - 20% ACN + 0.1% CH2O2; quaternary pump mobile phase: A - 95% H2O + H3PO4 and B - MeOH, flow: 0.4 and 1.0 mL min-1 for binary and quaternary pump, respectively; injection volume: 900 µl. Range for the analysis of antibiotics in water: 25 to 500 ng L-1. No residues of the three antimicrobials (NOR, ENR, CIP) were found in the water samples analyzed. It is believed that fluoroquinolones molecules are strongly adsorbed negatively charged on soil and depth sediment of rivers/lakes preventing its dispersion in water. Therefore, it was concluded that these drugs, which are currently applied in production of broiler chickens in Brazil (NOR, ENR, CIP), presents a low potential for contamination of water surface.

Support: FAPESP

Keywords: Contamination, Drugs, Environmental/Water, Liquid Chromatography/Mass Spectroscopy

Application Code: Environmental

Methodology Code: Liquid Chromatography/Mass Spectrometry
The identification of emerging contaminants such as those derived from pharmaceuticals and personal care products are crucial for the prevention of potential human health risks and environmental damage. Tandem mass spectrometry techniques are employed for the determination of unknown glucuronide metabolites based on the characteristic fragmentation of glucuronic acid. Glucuronide metabolites form in humans when a glucuronic acid is attached to a compound to facilitate its elimination. Thus the determination of glucuronide metabolites provides insight into unknown, potentially toxic compounds to which humans may be exposed.

Grab samples were collected in and around the city of Chicago (natural water and wastewater influent and effluent) and extracted for constant neutral loss analysis. Short, sequential mass ranges were analyzed using several injections. We have determined the molecular mass and retention times of at least 30 glucuronide metabolites over a 450 Dalton mass range using a triple quadrupole mass spectrometer interfaced to an HPLC system thus far. Accurate mass measurements of glucuronide metabolites were carried out with a quadrupole time-of-flight mass spectrometer.

Of particular interest is a glucuronide metabolite (403.0856) that is isobaric with the (M-H)- ions of the glucuronide metabolites of resveratrol (403.1030) and bisphenol A (403.1393) that was detected in Lake Michigan and effluent water from a treatment plant. Product ion spectra from the de-glucuronidated fragments have yielded little information toward an identity. Obtaining high resolution product ion spectra will be the next step in the determination of the empirical formula and structure of this and other glucuronide pollutants.

Keywords: Environmental/Water, Identification, Liquid Chromatography/Mass Spectroscopy, Tandem Mass Spectrometry
Environmental: Water

A Single Calibration Method for Water And Soil Samples Performing EPA Method 8260

The United States Environmental Protection Agency (USEPA) Method 8260 is used to determine Volatile Organic Compounds (VOCs) in a variety of matrices. These matrices can vary from water to soil to sludge. In order to determine VOCs in the assorted matrices, the sampling and analysis system needs to be calibrated as closely to the matrix as possible. Therefore, USEPA Method 8260 requires a separate calibration for waters, soils and extractions. This poster will demonstrate a patent pending automated water sampling mode using the soil sampling station of the autosampler thus eliminating the need to have separate calibrations for waters and soils.

Abstract Text
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Keywords: Automation, Purge and Trap, Volatile Organic Compounds, Water
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
A photochemical vapor generation system coupled to an inductively coupled plasma optical emission spectrometer (ICP-OES) was used for the determination of inorganic mercury in high salinity water arising from offshore petroleum platforms, i.e., produced water (PW). Analytical conditions, including low molecular weight acid type and concentration, UV exposure time and solution pH were optimized. The system comprised a 17.4 W UV grid lamp for photogeneration along with two tandem gas/liquid separators to minimize aerosol transport, as described in Figure 1. A 7.50% v/v solution of formic acid, pH 2.0 and exposure time of 90 s using continuous-flow mode permitted inorganic mercury to be determined based on external calibration using a matrix matching approach. A limit of detection and limit of quantification of 0.12 µg L \(^{-1}\) and 0.40 µg \(^{-1}\), respectively, were achieved, sufficient to use the proposed methodology for verification of laws imposed by the Brazilian National Council of Environment (CONAMA). Recoveries of spikes of Hg (II) added to samples were between 66 and 120% at the 10 to 40 µg \(^{-1}\) level.

**Abstract Text**

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Emerging contaminants are chemicals that are being detected with increasing frequency and at unexpected concentration levels in our environment. Pharmaceuticals, personal care products, and artificial sweeteners are among the many types of chemical classes that are passing through wastewater treatment plants and into water supplies around the world. Caffeine is now being monitored as a ground water tracer. Although these compounds are in trace concentrations, it is unknown what impact they will have on long term exposure. New extraction procedures will be necessary to monitor these compounds. Solid phase extraction (SPE) cartridges were selected for drinking water samples as the samples have little sediment. In addition, lower capacity cartridges were used as the analyte concentrations are low. Faster concentration of the extracts is achieved due to the lower extraction volume and reduced retentive water. These chemically disparate group of emerging contaminants were best extracted with custom formulated multi-modal cartridges over mono-modal varieties. In this presentation the results from using multimodal cartridges for selected pharmaceuticals, personal care products and non-nutritive sweeteners in drinking water using automated solid phase extraction instrumentation and automated evaporation will be shown.

Keywords: Automation, Environmental/Water, Solid Phase Extraction
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Abstract Text
Fluoride ion contamination for drinking water has caused serious health problem such as mottled teeth in developing country. Therefore, the development of simple analysis for fluoride has been desired. Colorimetry is one of the effective on-site analyses, because it is unnecessary analytical instrument for the 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 a selective chromogenic reagent for fluoride. Moreover, the 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 visibility because these ON-OFF color reactions are not able to be binarized fully. For this reason, in this study, we attempted to improve the visibility of this visual analysis by the assistance of image processing technology.
An accurate measurement of fluoride in drinking water and environmental samples is important for health care and environmental applications. Solid-state fluoride selective electrode technique based on the LaF3 crystal has been the standard method for fluoride measurement. However, this electrode is expensive and inconvenient for miniaturization. Polymeric membrane potentiometric fluoride ion-selective electrodes based on aluminum tetraphenylporphyrin (Al(III)TPP+) were recently reported with good selectivities and detection limits. However, the limitation of these sensors is the interference from the ubiquitous hydroxide ions. Thus, they require buffering the solution of interest at very acidic pH levels (pH ~3) and thus are not convenient for fast onsite measurements. We present here pulsed chronopotentiometric sensors to measure fluoride ions in water and environmental samples at the normal pH of the sample of interest. This technology utilizes ion-selective membranes that contain a lipophilic salt of the form R+R-. In these experiments, R+ is Al(III)TPP+ is used as a fluoride carrier and R- is tetrakis[3,5-bis(trifluoromethyl)phenyl] borate, TFPB- is used as the counterion. This method is explored for kinetic discrimination of the interfering hydroxide ions. Briefly, a current pulse is applied to cause the extraction of ions from the sample into the membrane. Low hydroxide ion concentration is depleted at the membrane surface and it should not contribute significantly to the change in the membrane potential. That is, the membrane potential will be mainly governed by the concentration of the more abundant fluoride ions. This enables direct detection of fluoride ions in drinking water at the action limit level (0.04-0.11 mM fluoride) at the natural pH of water ~ 7 (0.0001 mM hydroxide). Note that the more abundant ions in water such as chloride are discriminated by the selectivity of Al(III)TPP+. Thus, both kinetic and thermodynamic advantages have been utilized.

Keywords: Electrochemistry, Electrodes, Environmental/Water, Membrane
Application Code: Environmental
Methodology Code: Sensors
Conductivity detection is the primary detection technique for ion analysis by suppressed Ion Chromatography. The suppressor plays an important role in detection by eliminating the influence of the eluent and sample counter ions thereby maximizing the response for fully dissociated species. For weakly dissociated species the response is dependent on the pKa or pKb and concentration of the respective species. The strongly dissociated species after suppression are fully in the ionic form and are easily detected. The weakly dissociated species such as boric acid on the other hand are not dissociated enough and hence are not easily detected by conductivity detection. Further a response versus concentration curve is non-linear for weakly dissociated species by suppressed conductivity.

We have recently introduced a new detector for ion analysis – The Charge detector (QD). In this concept an electrodialysis type unit is used to de-ionize a suppressed effluent stream containing analytes. By designing a near 100% current efficient electrodialysis unit, the approach results in a current signal that directly measures the transient species. Here we will be presenting the utility of the QD for analysis of both fully and weakly dissociated ionic species. We will also present usefulness of QD in analyzing organic acids, inorganic anions and inorganic cations in a variety of samples such as drinking water, wastewater, fruit juice, wine and beverage samples. Further we will show, QD when combined with suppressed conductivity detection, can be used as a confirmatory tool, or as a complimentary detector to provide additional analytical information.

**Keywords:** Beverage, Capillary Ion Analysis, Detector, Environmental/Water

**Application Code:** Environmental

**Methodology Code:** Other (Specify)
Environmental: Water

Evaluation of Microbiological Qualities of Tyume River Located in Amatole District, Eastern Cape Province, South Africa

Health risks associated with surface water use, either raw or treated, include infectious diseases predominantly caused by human and animal enteric pathogens, including viruses. This study assessed the microbial quality indices of Tyume River in South Africa over a 12 months period. Samples were transported in ice to the Applied and Environmental Microbiology Research Group lab at the University of Fort Hare and analysed within six hours of collection. Total coliforms, faecal coliforms and enterococci were enumerated using culture based methods while viruses were quantitatively detected using real-time PCR. Serotype-specific multiplex PCR assays were used to detect the epidemiologically important virus serotypes. Faecal coliforms and enterococci both exceeded the guidelines of 200 CFU/100 mL and 33 CFU/100 mL respectively for recreational water. Faecal coliform count also exceeded the 103 CFU/100 mL guideline for water used in fresh produce irrigation. Adenovirus was detected in 31% (22/72) of the samples (in concentrations ranging from 1.0×101 to 8.49×104 genome copies/L). Of these, 59% were positive for species C adenovirus and 18% for species F adenovirus. Prevalence of norovirus and rotavirus was 4%. Rotavirus concentrations ranged from 9×101 to 5.64×103 genome copies/L. Hepatitis A virus was detected in 13% of the samples in concentrations ranging from 1.67×103 to 1.64×104 genome copies/L. Enteroviruses were not detected. The presence of enteric viruses in the rivers is suggestive of the dynamics of the same in the host populations and that there is considerable risk of infection posed by the river water for either domestic or recreational purposes.

Keywords: Environmental/Water, Monitoring
Application Code: Bioanalytical
Methodology Code: Other (Specify)
### Session # 1400 Abstract # 1400-19

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**Abstract Text**

Newer USEPA VOC Methods have given laboratories more freedom with instrument parameters, allowing for flexibility that have enabled faster, more efficient analyses. In keeping with this trend, this poster will examine using alternative carrier and purge gases to achieve higher throughput for purge and trap environmental methods. The Atomx multimatrix autosampler will be used in conjunction with a GC/MS to achieve more efficient analyses.
Surfactants are a known interference cited in U.S. EPA and ASTM cyanide analysis methods. In total cyanide methods employing a preliminary distillation step surfactants cause foaming that can result in low recoveries. In MicroDist distillation foaming from surfactants degrades membrane performance resulting in a negative bias or complete loss of sample. Surfactants also interfere with newly approved U.S. EPA cyanide analysis methods employing gas diffusion in place of a distillation step. This poster will examine the effect of surfactant interference on NPDES wastewater samples analyzed for total cyanide content by ASTM D 7511. Analytical data will be presented to demonstrate the symptoms of surfactant interference and possible solutions to mitigate and overcome the interference.

Keywords: Environmental/Water, Instrumentation, Ion Chromatography, Surfactants
Application Code: Environmental
Methodology Code: Chemical Methods
Environmental laboratories performing GC-MS analysis of VOCs in drinking water, wastewater, groundwater, and soil and sediment samples require a versatile and reliable automation platform to transfer samples to purge-and-trap GC-MS system. Sample requirements cited in U.S. EPA methods directly affect the design and performance characteristics of VOC analysis autosampler workstations.

This poster will present information on a new water and soil sample processor that meets the operational needs of environmental testing laboratories and fulfills U.S. EPA hardware and quality control requirements. Design and performance characteristics of this new sample processor will be presented, along with representative data obtained on water and soil samples using single and tandem purge and trap sample concentrators following USEPA-approved methods.

Keywords: Environmental/Soils, Environmental/Water, GC-MS, Purge and Trap
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Samples for ion chromatography (IC) can be heavily contaminated with particulates. Particulates are deleterious to the performance of modern high performance chromatography equipment, occasionally causing clogs in injection valves or other high tolerance sections of the instrument. Clogs frequently cause shortened guard column lifetimes with the accompanying expense in time and money of removing and replacing consumables. Organic contamination of aqueous samples for IC is also an issue- the polymeric columns used for high performance ion chromatography are sensitive to organic compounds, tending to absorb the organic contamination irreversibly. Progressive loss of column retention capacity or increasing back pressure caused by swelling of the packing materials causes frequent, expensive, replacement of guard and analytical columns. The current work demonstrates fully automated methods to treat samples such as wastewater and ground water samples by filtration through submicron porosity filters to remove particulates, along with back flushing which significantly increases filter lifetime- to thousands of samples in some cases. In addition similar schemes used to remove organics from samples, dramatically lengthens the lifetime of the analytical columns. Back flushing of the sacrificial absorbent columns to wash off retained organics makes use of these trap columns practical, as they can be used for many sample cycles before replacement. Milk and milk products are treated this way, as well as aqueous samples contaminated with organics.
Perchlorate salts are used as “rocket fuel” in electroplating and other industries; yet, in 1999 (C&EN article) it was scientifically proven to inhibit the human thyroid gland’s absorption of iodine — which, in turn, may cause thyroid-related diseases. Perchlorate detection/analysis in varying water matrices can be a challenge. How do we overcome the obstacles? Bromide is a naturally occurring element in water; however, when water utilities combine ozonation with UV light to disinfect our drinking water, this common process converts bromide to bromate — a known carcinogen that is regulated by USEPA. How precise can its detection get? These contaminants, as well as simple and robust analysis of them, will be discussed at length.

**Keywords:** Analysis, Environmental Analysis, Environmental/Water, Ion Chromatography

**Application Code:** Environmental

**Methodology Code:** Liquid Chromatography
Ion Chromatographic Separation of Divalent Cations by Lewis Base-Coated Zirconia Stationary Phase Column

A zirconia-stationary phase column functions as an anion-exchange column under an acidic elution condition at pH < 6. The column changes cation-exchange column by coating a Lewis base such as phosphoric acid or citric acid on the zirconia stationary phase. For example, when dynamically coating citric acid onto the zirconia stationary phase (Zirchrom-PHASE), the column can separate several divalent cations (\( \text{Mg}^{2+}, \text{Ca}^{2+}, \text{Mn}^{2+}, \text{Zn}^{2+}, \text{Cd}^{2+} \)) by acidic eluent (nitric acid, or acetic acid). The elution order of cations on zirconia column was similar to that on typical cation-exchange separation using a cation-exchange resin column. Interestingly, the citric acid-coated zirconia column enhanced the retention for analyte cations with rise in the column temperature (Fig. 1)[1-2]. Even when using the columns coating other Lewis bases (e.g., phosphoric acid, tartaric acid, succinic acid), the similar tendencies of the retentions was obtained.

Keywords: HPLC Columns, Ion Chromatography, Metals, Water
Application Code: Environmental
Methodology Code: Liquid Chromatography
The security and purity of low density polyethylene packaged water for human consumption during their shelf life was studied. Assessment of protective ability of thin film polyethylene packaging material of twelve brands of sachet water randomly obtained in Mushin area of Lagos state was carried out by challenging the samples with Escherichia coli, methyl violet solution and dual purpose kerosene (DPK) respectively to estimate the extent it protects the content (water) from being contaminated by microorganisms, solute and vapors.

Five of the twelve brands (41.7%) were contaminated with microorganisms most likely during production. A brand was contaminated with Escherichia coli while another one with Schistosoma spp. Three other brands were contaminated with Shigella spp. Microbiological challenge test result indicated presence of challenge organism in one brand within two weeks and in three brands at fourth week. At third day of exposure to DPK, eight brands had smell and tastes of DPK, all the brands were permeated by DPK at seventh day of exposure. Only one brand was penetrated by dye (methyl violet) at first week of immersion and five other brands at fourth week of immersion. The study shows the need for the improvement of the quality of the packaging materials for sachet water and also the need to reduce its shelf life.
Environmental: Water

An Inexpensive Semi-Automated Method for On-Site Process Monitoring of Total Trihalomethanes and Total Haloacetic Acids in Drinking Water

The disinfection of drinking water is important to protect public health against water borne diseases. However, water chlorination process leads to the formation of disinfection by-products (DBPs) that pose adverse health effects. The two major classes of DBPs are haloacetic acids (HAAs) and trihalomethanes (THMs). As the stricter Stage 2 Disinfectants and Disinfection By-Products (D/DBPs) rule gone fully in effect in the United States by October 2013, many water utilities that were barely meeting the previous, less restrictive regulations, will likely not be able to maintain compliance.

Water treatment operators need a cost-effective method for process control. The absence of an affordable on-site method for THMs and HAAs process control needs to be addressed. The standard methods developed by United States Environmental Protection Agency (USEPA) for quarterly compliance monitoring of THMs and HAAs have low method detection limits and high accuracy and precision, however, they are expensive to maintain and operate.

Disinfection by-product-rapid response (DBP-RR) kit was developed for on-site process control of Total THMs and Total HAAs from 20 to 120 ppb in drinking water using standard addition. Capillary membrane sampling (CMS) device is used to separate the THMs from HAAs. The method is followed by a batch reaction of THMs and HAAs with nicotinamide and base and detection using a handheld fluorescence detector. The sampling and data treatment processes are automated with software to minimize human error. This presentation will focus on the development and real-world application of DBP-RR kit using standard addition calibration.

Keywords: Environmental/Water, Fluorescence, Portable Instruments, Process Monitoring

Application Code: Environmental
Methodology Code: Portable Instruments
Environmental: Water

The contamination of heavy metal through the discharge of industrial wastewater is a worldwide environmental problem and a threat to human health. Removal of these contaminants from water is a challenge. More environmental friendly and effective treatment methods are in urgent need. The major objective of this study is to develop new bioadsorbents by using agricultural byproducts (ABPs). Processed rice hull was studied as biosorbent for heavy metal removal from water. The elements tested include most of the EPA regulated and CCL listed ones, they are: Al, As, Sb, Ba, Be, Cd, Cr, Co, Cu, Pb, Mn, Mo, Ag, Sr, Se, Te, Ti, V, U, Zn, total of 20 elements. The elements concentrations were detected by inductively coupled plasma-mass spectrometry (ICP-MS) method. Very promising results have been obtained. The processed rice hulls removed most of the elements efficiently except the negatively charged oxyanions. The removal of Be, Al, Co, Ni, Zn, Sr, Ag, Cd, Ba and Tl ions increased as the pH increasing while the removal of V, Mo and Pb decreased as pH increasing. Electrostatic attraction between negatively charged rice hull and positively charged metal ions is assumed the dominate removal mechanism. Therefore, there was no removal for negatively charged oxyanions such as As, Se and Sb ions. The processed rice hull was successfully employed for metal elements removal in real water sample matrix.

Abstract Text

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Environmental: Water

Using Agricultural Byproduct Rice Hull as Biosorbent to Remove and Recover Metal Ions in Water

Abstract Title

Primary Author Yongbo Dan
Missouri University of Science and Technology

Co-Author(s) Honglan Shi

Abstract Text

The contamination of heavy metal through the discharge of industrial wastewater is a worldwide environmental problem and a threat to human health. Removal of these contaminants from water is a challenge. More environmental friendly and effective treatment methods are in urgent need. The major objective of this study is to develop new bioadsorbents by using agricultural byproducts (ABPs). Processed rice hull was studied as biosorbent for heavy metal removal from water. The elements tested include most of the EPA regulated and CCL listed ones, they are: Al, As, Sb, Ba, Be, Cd, Cr, Co, Cu, Pb, Mn, Mo, Ag, Sr, Se, Te, Ti, V, U, Zn, total of 20 elements. The elements concentrations were detected by inductively coupled plasma-mass spectrometry (ICP-MS) method. Very promising results have been obtained. The processed rice hulls removed most of the elements efficiently except the negatively charged oxyanions. The removal of Be, Al, Co, Ni, Zn, Sr, Ag, Cd, Ba and Tl ions increased as the pH increasing while the removal of V, Mo and Pb decreased as pH increasing. Electrostatic attraction between negatively charged rice hull and positively charged metal ions is assumed the dominate removal mechanism. Therefore, there was no removal for negatively charged oxyanions such as As, Se and Sb ions. The processed rice hull was successfully employed for metal elements removal in real water sample matrix.

Keywords: Elemental Analysis, Elemental Mass Spec, Environmental/Water, ICP-MS

Application Code: Environmental

Methodology Code: Atomic Spectroscopy/Elemental Analysis
Rapid, on-site detection is critical for assessing health risks and locating ideal sampling sites. Portable GC-MS allows for the detection and identification of potentially hazardous contaminants in the field, however many sample matrices still require some level of sample preparation prior to analysis. Environmental water sample analysis poses a challenge due to high levels of sediment and debris. Purge and trap or headspace analyses are only useful for the extraction of purgeable, highly volatile organic contaminants (VOCs). The extraction of semi-volatile or non-purgeable contaminants is traditionally performed using liquid extraction with a non-polar organic solvent, but this is not practical in the field. Various types of solid-phase extraction allow for simple extraction and enrichment of non-polar analytes from aqueous matrices. Stir Bar Sorptive Extraction (SBSE) utilizes a layer of polydimethylsiloxane (PDMS) coated on a magnetic stir bar to simultaneously agitate and extract an aqueous sample. The relatively large quantity of the solid phase allows for quantitative extractions of analytes with log Kow values greater than ~2.6. The Stir Bars can then be thermally desorbed directly into a GC-MS system. This study will evaluate the use of SBSE in combination with a rugged, portable GC-MS to detect semi-volatile environmental pollutants (alkylphenols), chemical warfare agent simulants (methyl salicylate, tributyl phosphate), and explosives (TNT, RDX) in water samples. Typical sample volumes are 10 – 20 mL and resulting method detection limits are in the low ng/mL range.

Keywords: Environmental/Water, Gas Chromatography/Mass Spectrometry, Portable Instruments, Solid Phase E
Application Code: Environmental
Methodology Code: Portable Instruments
A Suppressor is a key component of an Ion Chromatography System with conductivity detection. The role of the suppressor is to remove the contribution from the background conductivity of the eluent by exchanging the counterions to the eluent for hydronium or hydroxide ions. The net effect is a decrease in the background conductivity of the eluent while enhancing the signal particularly for fully dissociated ionic analytes. Overall this process improves the signal to noise ratio and the detection limits thus leading to its broad applications for ionic speciation and quantitation in various sample matrices.

Recently we introduced a new generation of Electrolytically Regenerated Suppressor (ERS). This suppressor is housed in a new hardware and has improved flow and dispersion characteristics, which makes it fully compatible with 4 micron, small particle size packed ion exchange columns. In this presentation, we will review the design of this new suppressor, its applications for ion analysis and its utility as sample pretreatment device. We will also show the performance of the device in conjunction with carbonate removal devices.

Keywords: Chemical, Environmental Analysis, Environmental/Water, Ion Chromatography
Application Code: Environmental
Methodology Code: Liquid Chromatography
Due to the modern industry development and some other human activities in the world, more and more water resources are classified as polluted. It was reported that roughly 11% of the world population does not have access to the safe water. So investigation of emerging contaminants in water body is a very crucial step for public health. Amount those impurities in water, pharmaceuticals and personal care products (PPCPs), have drawn great concerns. There are several major sources of PPCPs in the environment include: agribusiness, human activity (e.g., bathing, shaving, swimming), residues from hospitals, residues from pharmaceutical manufacturing (well defined and controlled).

This study was mainly focused on occurrence screening and investigation of PPCPs and degradation of some PPCPs in Missouri drinking water. In addition, the removal of PPCPs using different materials has also been studied. Solid phase extraction (SPE) combined with ultra-fast liquid chromatography – tandem mass spectrometry (UFLC-MS/MS) method was developed and used to fulfill the need of detecting ng/L level concentration of PPCPs and their degradates. Several oxidants such as chlorine, ozone, permanganate, peracetic acid, and absorption materials was studied for PPCP removal. The detailed experimental conditions, interferences, and results will be presented at the conference.

This study was supported by Missouri Department of Natural Resources, (MDNR) and environmental Research Center at Missouri University of Science and Technology.

Keywords: Analysis, Environmental/Water, Liquid Chromatography/Mass Spectroscopy, Solid Phase Extraction
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
Algae and bacteria are the primary sources of taste and odor problem in drinking water. Other sources such as wastewater and chemicals spills can also be a source of compounds that cause unpleasant tastes and odors. Some species of algae and bacteria produce these specific substances inside their cells. In the 1960's and 1970's, it was discovered that geosmin and 2-MIB (2-methylisoborneol) are produced by cyanobacteria as intracellular by-product, such chemicals can affect both groundwater and surface water. Geosmin (trans-1,10-dimethyl-trans-9-decalol) and 2-MIB (2-methylisoborneol) are two of the most common compounds liable of the unsightly taste-odor in environmental matrices. The aim of this work was to develop a simple, fast and sensitive analytical method for the determination of earthy and musty odor organic compounds in the environmental matrices using a Dynamic Headspace/Purge&Trap-Gas Chromatograph-Mass Spectrometry system. The environmental sample is directly placed in a standard 20 ml vial, heated and purged with a flow of an inert gas for a defined time. The inert gas sweeps the sample and transfers the compounds in a cold focusing trap where they are concentrated. Finally, the trap is heated up and desorbed in backflush by the carrier gas. The extraction parameters are investigated. All data will be reported including chromatographic parameters.
Oysters have an economic and as a food source for many communities around the world. They are often grown where large industrial complexes are situated and run the potential of being contaminated. A potential contaminant is vanadium which is found in concentrations in the hundreds of ppm in Venezuelan crude oil. Therefore a great concern is that crude oil from an accidental release could contaminate oysters beds. A series of experiments in a controlled laboratory were designed and implemented to determine the uptake of vanadium and subsequent release by oysters. This involved spiking aquariums with no vanadium (control), low concentrations (~20 ppm) and high concentrations (~200 ppm) as well oil. The aquariums contained eight oysters each and each aquarium was in duplicate. At set periods the oysters were removed, shucked, dried, digested (via microwave technology) and vanadium determined by previously established (quality control/quality assurance) inductively coupled plasma-optical emission spectrometry method. After a period of time, oysters were removed from contaminated water and placed in pristine water for several weeks before vanadium determinations. Results of the study will be presented showing the uptake and subsequent release of vanadium by oysters, and factors affecting the process.
Increased N-nitrosamine precursors (amines) resulting from personal care products, pharmaceuticals, algae growth, agricultural activities are accumulating in water supplies. Additional amines can result from the usage of ion exchange resins, coagulants, as well as other materials utilized for water treatment. These amines are an emerging health concern due to possible N-nitrosamine formation in drinking water. In this study, the N-nitrosamine formation potential by treatment of disinfectants, free chlorine (FC) and peracetic acid (PAA), in drinking water has been investigated by using an isotope dilution solid phase extraction (SPE) – gas chromatography – mass spectrometry (GC-MS) method. Eight isotope-labeled N-nitrosamine standards were spiked in water samples before SPE. SPE was conducted by following the EPA 521 standard method. N-nitrosamines in the prepared water samples were detected by GC-MS. The test results show the N-nitrosamine formation potential is significantly lower by treatment of PAA compared with those of FC in high ammonia water sources. The detailed experimental conditions and results will be presented at the conference.

This study is supported by the US EPA STAR program (grant # 83517301) and Missouri Department of Natural Resource.
N-nitrosamines are a group of highly carcinogenic disinfection byproducts (DBP) formed during drinking water treatment process. Amines are precursors of N-nitrosamines. Therefore the monitor and removal of presented amines in water source are very essential to prevent the formation of N-nitrosamines. Currently most analytical methods for determination of amines involve derivatization process, which may encounter problems such as poor reproducibility, low recovery, and time consuming.

In this study, a fast and sensitive method for quantitative analysis of seven selected amines as N-nitrosamines precursors of in drinking water was developed using ultra-fast liquid chromatography with tandem mass spectrometry (UFLC-MS/MS). Separation and quantification of selected precursors are achieved in 10 minutes per sample, and no extraction or derivatization process is needed. Detection limits are ranging from 0.02 to 5 µg/L, and good linearity was obtained over a concentration range of 0.1 to 100 µg/L. The developed method was applied to determine the selected precursors in drinking water systems in Missouri. Several amines were detected in the low µg/L level. Detailed results will be presented on the conference.

This work was supported by the Missouri Department of Natural Resources and Environmental Research Center at Missouri University of Science and Technology.

Keywords: Environmental Analysis, Environmental/Water, Liquid Chromatography/Mass Spectroscopy, Tandem
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
Ammonia Removal from Drinking Water System Using Zeolite

Many drinking water treatment systems, especially the small water systems those use underground water as water source, are facing problem of naturally-occurring high ammonia in their source water, which complicates the chlorination disinfection process. Regular chlorine dosage is not effective enough for disinfection of water. When a higher dose of chlorine is applied, high levels of toxic disinfection byproducts (DBPs) are formed with the levels above the EPA DBP regulation limit. To solve these problems and to improve the drinking water quality, it is critically important to develop practical and effective technologies that remove ammonia from source water before chlorination. The major objective of this study is to develop methods to remove ammonia from source water by using different types of adsorbents. Naturally occurring zeolite was studied for ammonia removal from water. HACH TNT test kit and total nitrogen analyzer were used to monitor the ammonia concentration. The test results indicated that the zeolite can effectively remove ammonia from water rapidly. The removal efficiencies under different dosages, pHs, temperatures, and reaction times has been evaluated. The detailed experimental conditions and the results will be presented at the conference. This study is supported by US EPA STAR program (grant # 83517301) and Missouri Department of Natural Resource.

Keywords: Absorption, Environmental/Water, Spectrometer, Water
Application Code: Environmental
Methodology Code: Portable Instruments
Environmental impact caused by mining has become a worrying issue because the magnitude of the damage in the affected areas. In the north of Colombia has been registered a significant increase in mining activities, including the opening of many gold mines, artisanal and small-scale. Mercury is widely recognized as a highly toxic pollutant to humans, and there are many studies about Hg used in gold extraction processes. Discharges of mining wastes into aquatic systems have led to increases of Hg concentrations to levels that are higher than those considered safe for people. For this reason, in this study Hg concentrations and a method of Hg removal were evaluated for the treatment of a mining effluent.

Hg-Total concentrations in all samples collected, before and after of the treatment, were determined by CVAAS in a Thermo electron Model 3000iCE Spectrometer. A Flat Plate Collector (FPC) and a Compound Parabolic Concentrator (CPC) were used to treat samples of water. Acid and alkaline conditions, concentration of TiO\textsubscript{2} and the cumulated intensity of solar radiation were considered as factors in the process. According to results, maximum permissible limit for Hg in surface water was exceeded by concentrations in water samples taken from a mining effluent. Removal rates of Hg were higher using the CPC in comparison with results obtained in the FPC. In general, results showed that the removal of Hg of up to 93% was obtained, and pH was the most influential factor in the process (P<0.05). Finally, low operating costs of the photocatalytic process, facility of installation and operation, and ability to reuse the TiO\textsubscript{2} catalyst are factors that influence that this can be regarded as a promising technique for the control and treatment of mining effluents.

Authors are grateful to Colombian Institute for the Development of Science and Technology "Francisco Jose de Caldas" COLCIENCIAS and to University of Cordoba, Colombia, for the agreement 549-2012.
Determination of Mercury (Hg) in Water by Hand-Held, Portable Cold Vapor Atomic Fluorescence Spectrometry

Mercury concentrations in water were determined using a hand-held, portable, cold vapor atomic fluorescence analyzer that is traditionally used for sampling ambient air. The samples were prepared using a procedure adapted from EPA method 1631 Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry. The mercury masses are expressed in total [micro]g measured, with variable volumes of total water used, expressed in mL. Mercury concentrations were successfully determined in samples from 10ng/m3 to 0.4[micro]g/m3. Testing without the use of a gold trap concluded after approximately one hour. This is displayed by plotting data points over time, which produced a graph that resembles an exponential decay. Additionally, the portability of the instrument allows for measurement of mercury in different work stations, as well as in the field.

Keywords: Atomic Emission Spectroscopy, Detection, Environmental Analysis, Mercury
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Gemfibrozil, Ibuprofen and Triclosan Profiling in Tuscaloosa Waste Water Treatment Facility Using SPE and HPLC Analysis

Profiling of three major pharmaceuticals in the waste water effluent samples obtained from Tuscaloosa Wastewater Treatment Plant, Tuscaloosa, Alabama, was undertaken during dry and wet seasons. Water samples were extracted for three specific pharmaceuticals: Gemfibrozil, Ibuprofen and Triclosan, using Oasis HLB Cartridges. The concentrates were solvent exchanged with methanol and analyzed by reverse phase HPLC/UV using XterraMS C18-Column with 10 micron stationary phase. Triclosan was found in the range of 25 - 50 ppb range during rainy season, mainly in the primary treated effluent samples. Gemfibrozil was detected in trace amounts in some samples. Data obtained over a period of six months using sixty samples are compared and presented.

Keywords: Environmental/Waste/Sludge, Environmental/Water, HPLC, Solid Phase Extraction
Application Code: Environmental
Methodology Code: Liquid Chromatography
The influence of fertilization on water sources near corn and soybean agricultural fields will be presented in this study. Water runoff carries chemicals in fertilizers and pesticides (often used on crops) to larger bodies of water leading to an accumulation of these chemicals in the water supply. The addition of pesticides and fertilizers to the aquatic environment can be catastrophic because most pesticides are toxic, affecting the health and survival of aquatic organisms and living creatures using the water supply. The types of chemicals found in fertilizers and pesticides are: ammonium nitrate, ammonium sulfate, potassium chloride, phosphorous, and nitrogen. One fertilizer compound studied, nitrogen, encourages plant growth; however, when plant growth is too large, the algae of the water system will grow and reproduce too quickly. The decay of algae leads to reduced oxygen levels in the water essentially affecting the entire ecosystem of the pond negatively (death of plant and animal life).

The area being studied includes animals such as a family of albino deer and their possibly contaminated water source. To assess the contamination caused by pesticides and fertilizers, the bodies of water around these farm lands will be tested for nitrate, phosphorous, chloride, ammonium nitrate, pH and heavy metals. The nitrate and phosphorous levels will be analyzed via Ultra Violet–Visible spectroscopy (UV-Vis), chloride and ammonium nitrate will be analyzed using titration, and the heavy metals analyzed using Inductively Coupled Plasma Spectrometry (ICP). This study will help conclude if the fertilizers and pesticides used on agricultural fields are affecting the water bodies surrounding them.
Only 3% of water on Earth is in the form of drinking water. Out of this 3%, 2% is in the form of ice. Thus, only 1% of the drinking water on Earth is readily available for consumption. As populations grow, demand for drinking water is increasing. In order to provide more drinking water, the need to analyze pollutants in water, recycle water, and desalinate seawater is on the rise. This presentation will demonstrate successful use of ion analysis techniques for measuring pollutants and micro nutrients such as Nitrite-N, Nitrate-N, Phosphate-P and Ammonium ion in various sources of drinking water (surface water, ground water, seawater). This poster presents simple and robust method for micronutrients analysis by suppressed conductivity tandem with direct UV/Vis detection.

Keywords: Environmental Analysis, Environmental/Water, Ion Chromatography
Application Code: Environmental
Methodology Code: Liquid Chromatography
Environmental: Water

Removal of Metals from Aqueous Solution Using Functionalized Magnetic Nanoparticles

The use of magnetic nanoparticles as a support material has drawn increased interest due to their rapid and easy separation ability and recovery from the reaction medium in an external magnetic field. A previous treatment of a silylating agent, containing the required functional groups is a method for the production of pendant organic chains covalently bonded to the support material. By applying such methodology, the magnetic nanoparticles are progressively used in many activities such as biotechnology (1), cation removal (2), adsorption of organic compounds (3) and etc.

Thioglycolic acid molecule embraces two basic centers, oxygen and sulfur, and can easily be grafted on Fe3O4 particle surface previously modified with the precursor silylating agent. Modification with thioglycolic acid gives the nanoparticle the ability to form stable complexes with several metals.

The main goal of this study is the preparation of very active adsorbent material to remove heavy metals from water. In this study, thioglycolic acid was immobilized onto Fe3O4 nanoparticle surface using 3-aminopropyltriethoxysilane (APTES) as precursor silylating agent. This new surface displayed a chelating moiety containing nitrogen, sulfur, and oxygen basic centers which are potentially capable of extracting cations from water solution. Experimental conditions for effective sorption of metal ions were optimized with respect to different experimental parameters using batch method in detail.

References

Keywords: Environmental/Water, Materials Science, Nanotechnology, Water
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Although nitrate has been determined routinely in most rain water surveys due to the concerns about the causes of acid rain and the eutrophication of surface water, measurements of nitrite in snow and other atmospheric condense phases are scarce. Recently, we have reported on an ion-pair HPLC method for the determination of nitrite and nitrate in dew, and rain waters (Zuo et al., 2006). In this presentation, we report on a more sensitive hydrophilic interaction HPLC method for the simultaneous determination of nitrite and nitrate in snow and other environmental samples.

Quantification was carried out by the peak area integration method using a Waters HPLC 2695 Module with UV-visible detection at 215nm. A clean separation of analytes was achieved in less than 8 minutes. Limits of quantification (LODs) of 5.0 ppb for nitrate and nitrite were obtained. This developed method has been successfully applied to the analysis of snow samples collected in the Southeastern Massachusetts. The concentration of nitrate and nitrite has been found in the range from undetectable to as high as 3.5 and 0.90 ppm, respectively. The method proved a sensitive, accurate, and cost-effective technique for the analysis of trace level nitrate and nitrite in snow, drinking water and other environmental water samples.

Keywords: Environmental/Water, HPLC, Separation Sciences, Trace Analysis
Application Code: Environmental
Methodology Code: Liquid Chromatography
Analyses of trace metal elements are generally performed by expensive instrumental methods, such as inductively coupled plasma-mass spectrometry (ICP-MS) and graphite furnace atomic absorption spectrometry (GFAA). In addition to the high cost, the instruments are not always available in many cases. These methods are also not suitable for in field detection. This study developed a novel technology to pre-concentrate the heavy metal elements in environmental samples by solid phase extraction, and then detect the elements by a rapid and economic method. A large volume of water sample was extracted with a cartridge that packed with specific adsorbent, and then the metal elements were eluted with a small volume of solvent. The metal elements in the extracted samples were analyzed by using HACH test kits, which are rapid and less expensive. ICP-MS method was used to validate the newly developed method. The optimized experimental conditions and the method suitability for wastewater and drinking water samples will be presented on the conference.
The presence of an increasingly complex array of pharmaceuticals and personal care products (PPCPs) in water bodies throughout the world is placing a greater demand on techniques used to screen for these compounds. Using a high resolution accurate mass ToF screening technique employing MS\(^{E}\) where high and low energy switching enables the acquisition of precursor and fragments ion in a single injection, provides information rich data that can be used to reduce the large number of false detects that arise from the use of accurate mass alone. In combination with an integrated scientific information system, the ability to screen for the presence of PPCPs, their adducts and potential metabolites in a single injection is now possible in a routine laboratory.

Owing to the wide chemical diversity of the compounds described as PPCPs, the extraction and separation of the many classes and structures pose a major analytical challenge. Depending on detection level required, two alternative approaches could be taken for sample preparation. The first involves simple filtration followed by large volume injection of the water sample. If lower levels of detection were required, a method employing mixed mode solid phase extraction was used. UPLC separation on a 2.1 x 100 mm HSS T3 analytical column (1.7um) was found to provide optimum chromatographic conditions to resolve the range of chemically diverse PPCPs.

**Keywords:** Data Analysis, Environmental/Water, Liquid Chromatography/Mass Spectroscopy, Time of Flight MS

**Application Code:** Environmental

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
In this presentation, the spectrophotometric determination of sulfate by two indirect methods – one involving the use of a barium/chromate reagent, the other involving formation of a chelate of barium ion and Sulfonazo III – will be investigated, with an eye toward comparison of the two methods and their application to the determination of sulfate in abandoned mine drainage (AMD). The purpose of this investigation is to develop a method or methods for sulfate determination in AMD that are portable (i.e., can be used on site, at the sampling point), more rapid than traditional gravimetric methods, inexpensive, and can be scaled down in size to minimize waste generated by the use of barium- and chromate-containing reagents, as both species are considered toxic and must be disposed of according to proper protocols. The chromate method relies upon the solubilities of barium chromate and barium sulfate, with precipitation of sulfate as its barium salt and colorimetric detection of liberated chromate at 365 nm. The Sulfonazo III method involves addition of excess barium ion to the sample solution and chelation of excess barium ion in the solution after precipitation of sulfate as BaSO₄.

Sample collection and preparation protocols, and details of the analytical determination of sulfate by the chromate and Sulfonazo III methods, will be presented and discussed, as will results of the sulfate determinations, calibration data and results, and future directions for this research.

Keywords: Environmental/Water, Method Development, Spectrophotometry, UV-VIS Absorbance/Luminescence

Application Code: Environmental
Methodology Code: UV/VIS
Determination of 16 Environmental Protection Agency Polycyclic Aromatic Hydrocarbons in Water Samples via Solid-phase Nanoextraction and Gas Chromatography - Mass Spectrometry

Polycyclic Aromatic Hydrocarbons (PAHs) are an important class of targeted pollutants that derives from natural and mankind activities involving incomplete combustion of organic matter. The relevance of PAHs monitoring in the environment is well-known. Contamination with some PAHs might be harmful to human health due to their mutagenic and/or carcinogenic nature. A primary route of human exposure to PAHs is the ingestion of contaminated water. The research presented here involves a novel extraction procedure for PAHs in water samples called solid-phase nanoextraction (SPNE). When combined to gas chromatography – mass spectrometry (GC-MS), SPNE provides excellent analytical figures of merit. PAHs extraction is performed by mixing 500 [micro]L of water samples with 950 [micro]L solution of 20 nm gold nanoparticles. After mixing and centrifugation, the precipitate is treated with 2 [micro]L of 1-pentanethiol, 48 [micro]L of methanol and 50 [micro]L of n-octane. After shaking for 5 mins, the n-octane phase is analyzed via GC-MS. The entire extraction procedure takes approximately 20 mins per 12 samples. The simplicity of the extraction procedure, the small sample volume and the minimal consumption of organic solvents make SPNE environmentally friendly, cost effective and well suited for the routine analysis of numerous samples.

Keywords: Environmental/Water, GC-MS, PAH, Solid Phase Extraction
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Mercury is a known environmental pollutant that can damage the brain, heart, kidney and lungs upon exposure. Mercury quantification using flow injection analysis (FIA) methods has been extensively studied, resulting in published methods with extremely low (sub-ppb) limits of detection. Herein, use of gold nanorods (Au NRs) functionalized glass substrates for FIA detection of mercury is explored. The UV/Vis absorbance profile of the Au NRs displays a strong surface plasmon resonance band that is sensitive to the refractive index of the surrounding medium as well as the aspect ratio (length/diameter) of the NRs. When the Au NRs are exposed to Hg(0), the amalgamation between Au and Hg(0) causes a reduction of the effective aspect ratio of the nanoparticles and a blue shift of their maximum longitudinal surface plasmon resonance (LSPR) absorption wavelength. Quantitative analysis is made possible due to the linear correlation that exists between the concentration of mercury and the wavelength shift of the maximum LSPR absorption wavelength. Herein, we demonstrate that the immobilization of Au NRs onto a glass substrate is advantageous not only for its use as a portable sensor but also to preserve the physical integrity of the nanoparticles. This work is funded by the U.S. Department of Energy (DE-SC0004813).
TOC measurement is of vital importance to the operation of water treatment due to organic compounds comprising a large group of water pollutants. TOC has been around for many years, and although it is a relatively simple analysis in theory, operational efficiency is paramount. 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 test ground water and waste water samples showing a >25% improvement in laboratory efficiency following Standard Method 5310B and US EPA Method 415.1 guidelines.
The price of honey varies from one region to another. Unscrupulous traders may label honey containers purporting to come from regions known to produce high quality honey in order to get higher income from the sale of honey. The study aimed at identifying markers specific to Kenyan honey from different geographical regions so as to develop a fast reliable profile which can be employed in the geographical profiling of honey. Volatile organic compounds in honey from various regions of Kenya were extracted using solid Phase extraction method and subsequently analyzed using Gas chromatography- Mass spectrometry for separation and identification of volatile organic components. The volatile organic compounds identified in honey were esters, carbonyl compound (ketones and aldehydes), carboxylic acid and hydrocarbons. It was established in this study that the presence or absence of certain volatile organic compounds and the variation in their concentration can be used to classify honey from different geographical regions.

**Keywords:** Analysis, Extraction, Gas Chromatography/Mass Spectrometry, Natural Products

**Application Code:** Food Science

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Essential oils (EO) are widely used as natural flavoring material in food industries. The quality assessment of EO is of primary importance for both consumers and industries, not only to assure production consistency but also to confirm the natural origin and exclude possible adulterations. The common analytical approach for natural origin assessment is to determine the ratio between some chemical constituents characteristic of the product, or to determine specific biomarkers that prove either the adulteration or authenticity of the food. In the case of EO, adulteration phenomenon can be investigated referring to the chiral nature of several markers. In fact, chiral compounds from natural origins are usually present as a predominant optical isomer and the enantiomeric ratio is often correlated to the geographical origin of the product. On the contrary, the presence of a racemic mixture typically indicates synthetic origin or adulteration process. The use of chiral fast gas chromatography is very effective in the EO quality control, with the possibility to significantly shorten the analysis time for higher sample throughput. For this purpose, a new generation of chiral stationary phases as substituted cyclodextrins are of particular interest for routine analyses in fast enantioselective gas chromatography. Chiral short and narrowbore columns permits to significantly reduce the analysis time, providing the resolution required for enantiomers separation. Fully automated static headspace technique coupled to fast GC-TOFMS offers an easy and effective method for fast authenticity assessment of different type of food or beverages, with reduced sample handling and extended automation.

Keywords: Flavor/Essential Oil, Food Science, GC-MS, Quality Control
Application Code: Other (Specify)
Methodology Code: Gas Chromatography/Mass Spectrometry
Two species of spearmint are cultivated in the United States. In 2008, 1.09 million kilograms of spearmint oil were produced in the U.S. Forty-five percent of U.S. spearmint oil is used to flavor chewing gum. One 55-gallon drum of mint oil can flavor 5,200,000 sticks of gum or 400,000 tubes of toothpaste.

Sulfur compounds impart undesirable odors to essential oils. Volatile sulfur compounds have extremely low olfactory thresholds. Detecting, identifying, and eliminating sulfur compounds are important aspects of flavor and fragrance quality control.

This study examined the use of a Pulsed Flame Photometric Detector (PFPD) to locate and measure low-level sulfur compounds among the large number of hydrocarbon peaks present in a typical GC-FID chromatogram of neat spearmint oil. The analytical methodology employed in this rapid screening technique and chromatographic results will be presented.
Coffee and tea are heavily consumed in the United States as one of the main sources of antioxidants in the American diet. Antioxidants produced by the body prevent free radical damage by donating its own electron to terminate the chain reaction caused by these free radicals. An intake of antioxidants may help reduce oxidative damage to important cells and consequently help some health conditions such as coronary artery disease, high cholesterol, and diabetes. Different coffee and tea brands readily available at common grocery stores of different prices were analyzed for their antioxidant capacity and stability using the Trolox equivalent antioxidant capacity (TEAC) method. In this experiment, four common medium-French roast coffee and four organic (non-organic) green tea products were carefully selected. The antioxidant capacity proved to be dependent on brand type. Meanwhile, the antioxidant capacity proved to be decreased in a nonlinear pattern. Antioxidant capacity, irrelevant of brand, decreased quickly during the first few days and then at a much slower rate afterwards. Canned product had a faster decreasing rate compared to those freshly brewed products.
Vegetable oils are complex chemical mixtures, in which di- and triglycerides and free fatty acids are the main components, but a series of minor polar compounds is also present and their distributions are characteristic of different types of oils. Olive oil, an expensive oil with superior health benefits, is sometimes adulterated with lower priced oils. These results a need for authentication of oil samples by methods that are fast, straightforward, and accurate to determine their provenance, quality, and possible adulteration. Ideal analytical methods require minimal or no sample preparation. Available methods for characterization of vegetable oils include mass spectrometry along with other spectrometric techniques such as fluorescence, vibrational spectroscopy or nuclear magnetic resonance spectrometry. Extraction protocols involving liquid-liquid and/or solid-phase extraction are often required prior to reported mass spectrometric measurements.

In the present work, the performance of different mass spectrometry-based methods has been assessed for the direct analyses of virgin olive oil for quality purposes. Different experiments have been setup, including: (1) direct measurement of untreated olive oil using ambient mass spectrometric methods such as Low-Temperature Plasma Mass Spectrometry (LTP-MS) or paper spray mass spectrometry (PS-MS); or alternatively (2) the use of atmospheric pressure ionization mass spectrometry (direct infusion) using either electrospray or APCI sources, combined with a minimum sample work-up consisting of a simple olive oil dilution (from 1:10 to 1:1000) with appropriate solvents, or a quick liquid-liquid extraction to shift the measurement towards a specific part of the composition of the edible oil (i.e. polyphenol rich fraction or lipid/fatty acid profile).
The chemical composition of different beans species can vary according to genetic factors, origin geographic (soil, water, pesticides, insecticides and fertilizers) and climate. However, differences of the composition should be evaluated in cooked beans, since that it is the ingested form. Cooking procedures induce the production of desirable sensory properties in beans, such as sweet taste, cooked-bean flavor, and soft and mushy textures. Furthermore, the heating of the grains increases the protein digestibility and minimizes the effect of antinutrients, such as tannins. However, cooking causes considerable changes in the composition of numerous chemical constituents, including amino acids, vitamins and minerals. Therefore, the effects of the domestic cooking on essential elements (Ca, K, Mg, P and S) concentration of different beans species ([i]Phaseolus vulgaris[/i] L.) were investigated. Jalo, fradinho, rajado, rosinha, bolinha, black and common species were used in this study. The elemental determination was done by inductively coupled plasma optical emission spectrometry, after the sample digestion in a closed-vessel microwave oven, using diluted oxidant mixture (HNO₃+H₂O₂+H₂O). The accuracy of the entire proposed method was confirmed by analysis of a certified reference material (citrus leaves-NIST SRM1572). The certified values showed a good agreement at a 95% confidence limit. The interest elements concentrations in the different beans species were very close. An increase of the concentration was verified, depending on the specie and the element, ranged from 12 (rosinha, K) to 194% (black, Ca). Only in the fradinho and rajado species, the Ca concentration suffered a reduction (20%). Sulphur was the least affected by cooking. ACKNOWLEDGMENT: FAPESP(2012/11517-1)
Antioxidant Activities of Rosmarinus Officinalis L. Essential Oil Obtained by Hydro-Distillation and Solvent Free Microwave Extraction

The essential oils of Rosmarinus officinalis L. growing in a rural area within the Nkonkobe Municipality of the Eastern Cape, South Africa, were extracted using the solvent free microwave extractor (SFME) and hydro-distillation (HD) methods. The antioxidant and free radical scavenging activity of the obtained oils were tested by means of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH+) assay and ß-carotene bleaching test. In the DPPH+ assay, while the free radical scavenging activity of the oil obtained by SFME method showed percentage inhibitions of 48.80, 61.60 and 67.00%, the HD oil showed inhibitions of 52.20, 55.00 and 65.30% at 0.33, 0.5 and 1.00 mg/ml, respectively. In the ß-carotene bleaching assay, the percentage inhibition increased with increasing concentration of both oils, with a high antioxidant activity of the oil obtained through the SFME than through the HD method. The significance of this observation is discussed with respect to the properties of essential oils obtained using different methods.

Keywords: Natural Products
Application Code: Food Science
Methodology Code: Liquid Chromatography/Mass Spectrometry
Assessing the quality of processed food such as spiced sauces, is not easy using sensory panels since it is difficult to have a reference quality product when testing new production batches. In this study, it is proposed to use a gas chromatography based electronic nose to evaluate the quality of various batches of sauce. Nine samples of ranch sauce of different qualities or production dates (5 good, 2 bad and 2 blind samples) are tested with ULYS-S Electronic Nose. This instrument features one column (SE-54 type – length 25 m x I.D. 0.53 mm x phase width 1 μm) and two different detectors: one Flame Ionization Detector (FID) and one Flame Photometric Detector (FPD). Coupled to a database for chemical and sensory characterization by Kovats Index matching and an olfactometer, the system allows an in-depth analysis.

The chromatograms highlight significant differences of chemical composition. In particular, the chromatogram obtained with FPD detector allows to suspect that the presence of sulfur compounds in the bad batch is responsible for differences in sensory quality. Upon comparing the overall flavour profiles of the sauces, the various qualities are clearly differentiated, and the differences prove to be linked mainly with acetic acid, ethyl acetate, terpenes and sulfur compounds. The sensory evaluation via the olfactometer confirms the FPD measurements in that sulfurous odors were detected. Their origin could be linked with the presence of foreign spices like onion or garlic whereas this recipe should not contain these ingredients.

Keywords: Food Science, Gas Chromatography, GC, Quality Control
Application Code: Food Science
Methodology Code: Gas Chromatography
Food Science: Flavors

Quantification of the Bitterness Level of Olive Oils with an Electronic Tongue

In the best olive oils, bitterness, pungency and fruitiness are well-balanced sensory characteristics. The flavonoid polyphenols contribute to bitterness, resistance to oxidation, and are strong antioxidants that have been shown to provide beneficial health effects. In this study, it is proposed to assess the bitterness level of olive oil, usually evaluated by a sensory panel, by means of an electronic tongue. The ASTREE Electronic Tongue is based on liquid sensors which interact with chemicals present in the liquid sample and responsible for taste. Eight olive oils were analysed, six of them characterized by nine assessors and used for calibration set-up. Scores below 2 indicate a low bitterness, between 2 and 3 a middle bitterness and between 3 and 4 a strong bitterness. Compounds responsible for taste were collected by liquid-liquid extraction prior to the analysis. The instrumental measurements were computed using Principal Components Analysis to compare the global taste profiles of oils. The oils were clearly differentiated along an axis related with bitterness intensity.

To determine the bitterness intensity of the blind samples, a calibration model based on Partial Least Square regression was set up by correlating sensory panel results and instrumental measurements. A high level of correlation was obtained between the two techniques (correlation coefficient = 0.9585). It could be determined that B1 had a bitterness intensity of 2.55 and B2 of 2.57. The sensory panel scorings were in agreement: 2.5 for both B1 and B2 oils. This suggests that this technique can be successfully applied for routine analysis.

Keywords: Chemometrics, Food Science, Integrated Sensor Systems, Sensors
Application Code: Food Science
Methodology Code: Integrated Sensor Systems
Flavor profiles of imported and domestic beers by purge and trap thermal desorption GC/MS

Introduction
Flavor qualities of beers are greatly dependent on the volatile and semi-volatile organic compounds present in the liquid matrix and headspace aroma. These compounds are also used in the manufacturing process to obtain the desired physical properties of the product. Over time beer can develop off-odors or flavors due to a number of factors including the natural aging process, variations in storage temperatures or interactions with the container. The use of purge and trap coupled with Thermal Desorption GC/MS is used to show the different flavor profiles of various manufacturers’ brands over time.

Methods
Analysis were conducted using a commercial purge & trap apparatus and Thermal Desorption system coupled to a GC/MS operating in El ionization mode.

Results
Two beers, brand X (domestic) and Brand Y (imported) were analyzed by purge & trap GC/MS. The chromatograms were collected for each beer. Both beers were exposed to heat and light to simulate the aging process and re-analyzed and a comparison of the chromatograms was completed. The results showed that the concentrations of dimethyl sulfide, 2,3-pentadione, 2,3-butanedione and isoamyl acetate changed significantly over time. The increase of dimethyl sulfide concentration is thought to be a major contributor to off-odor and bitter taste qualities of old or stale beer.

Novel Aspects
TD/GC/MS is capable of monitoring a complete flavor profile of volatile and semi-volatile components in beer.

This work is funded internally by Scientific Instrument Services
Food and drink adulterations are an increasing concern worldwide. Many producers add water to milk aiming to increase milk volume, which results in a nutritional loss of the product by dilution. In order to “compensate” this loss and to maintain the physical characteristics of the milk, some substances, harmful to health (urea, formaldehyde and melamine), are added. Electrochemical techniques are easy to operate, allow the use of low cost materials for the fabrication of sensors and enable the quantification or discrimination of samples in a short time. Therefore, our work deals with the development of a voltammetric electronic tongue (VE-tongue), which is able to discriminate between adulterated and unadulterated milk samples with urea, formaldehyde or melamine. To this propose, cyclic voltammograms were recorded using the VE-tongue composed by three working electrodes (Pt, Au and Cu) immersed in milk samples adulterated and unadulterated. The cyclic voltammograms were obtained using the following potential program: from -0.5 to 1.6 V for Pt and Au working electrodes and -0.8 to 0.1 V for the copper electrode. All the current values obtained from the cyclic voltammograms were used as input for a non-supervised pattern recognition method (PCA) without any prior pre-processing and scaling. The score plot obtained by the VE-Tongue together with PCA allowed the implementation of a model that could distinguish between adulterated and unadulterated samples without any misclassification, demonstrating that device could be applied as an economical and fast procedure for discrimination of milk samples. Financial support: FAPESP, CAPES and CNPq.
Recently developed Shell-isolated nanoparticle-enhanced Raman spectroscopy (SHINERS) has significantly enlightened the potential applications of Raman spectroscopic techniques in analyzing chemicals rapidly and qualitatively. Here we reported the synthesis and optimization of similar core-shell nanoparticles for identification of trace amount of chemicals in mixtures, and even from raw samples. Different from pinhole-free core-shell nanoparticles, we explore the potential advantages of pinhole core shell particles through engineering the size and distribution of the holes on the surface of nanoparticles. In some instance, these nanoparticles have displayed high sensitivity and yet maintained resistance from interference. Nanoparticles composed of 50-120 nm gold core and 1-2nm SiO2 shell have been synthesized and purified, and applied for several chemical species in artificial mixtures as well as raw samples. Our results have demonstrated that we could easily detect ppm to ppb amount of target molecules that are commonly illegally used for in food industry (such as unpermitted pesticides and additives). Further aided with small sample separation units developed from collaborated groups, trace amount of such chemicals can be detected from raw samples obtained from local food markets. As we know that pinhole-free core-shell nanoparticles with very thin shells are very difficult to obtain, pinhole nanoparticles can be regarded as useful replacement to satisfy the strong requirements for fast and in situ sample analysis.
Free sulphur dioxide is active preservative in wine, and it acts as an antioxidant and antiseptic agent. Sulphur dioxide is found in wines in free forms (SO2 and HSO3-) and is bound to compounds that incorporate a carbonyl group, such as acetaldehyde. In this study, an automated SO2 free method is presented.

The method is based on the reaction between sulphur dioxide, p-rosaniline hydrochloride and formaldehyde. This method is designed to use optimal reagent concentration and volumes to be able to perform accurate results. The concentration of free sulphur dioxide in the sample is calculated automatically from the calibration curve.

The photometric method used correlates well with the FIA method. The new method is quick and performs accurately with both red and white wines. Concentration of samples varied from 0 to 46 mg/l, average being 28 mg/l. Samples (57) covering different types of wines were tested with correlation R2 being 0.9755. Precision for the red wine samples (N = 40) was calculated 1.8% within run, and 2.1 % between the runs.

Due to the bar-coded system reagents, this new automated SO2 free method is very quick and easy to use. Analysis of 60 samples takes 35 minutes with only about 10 minutes for daily calibration and analyzer start-up operations. From the same samples, additionally e.g. different sugars and acids, color and total SO2 can be run automatically. Compared to the FIA method, the photometric method requires only small volumes of reagents, thus being more economical and environmental friendly choice.

**Keywords:** Analysis, Beverage, Chemical, Sulfur

**Application Code:** Food Science

**Methodology Code:** Chemical Methods
Hot and spicy food has dramatically increased in popularity over the past 10-20 years. Capsaicin is the most abundant compound found in chili peppers giving them their fiery flavor. Capsaicin is formed when vanillylamine is coupled to a 10 carbon fatty acid through an amide linkage. However, there are other related compounds often called capsaicinoids. These compounds have the same vanillylamine group but differ by the associated fatty acid chain and are responsible for the perception of different heat profiles for different chili peppers. Some peppers are described as having a high initial flash of heat while other peppers are described by a long and late burning profile.

The work presented here, initially uses HPLC with UV detection to profile capsaicinoids extracted from several different chili peppers and commercially available hot sauces. Prep HPLC is then used to isolate individual capsaicinoids from the pepper extracts. Finally, a triple quadrupole MS system is employed to identify and quantitate the observed capsaicinoids.

Capsaicin was found to be the most prevalent capsaicinoid species in all of the studied matrices. Significant amounts of Nondihydrocapsaicin were found in a cayenne hot sauce and in thai chili pepper extract. Dihydrocapsaicin and Homodihydrocapsaicin were also identified in many of the investigated chili extracts and hot sauces but at lower levels. The typical concentration of these compounds were found to be in the ug/g range but varied widely among the different chili peppers and hot sauces.

Keywords: HPLC, Liquid Chromatography/Mass Spectroscopy, Natural Products, Prep Chromatography
Application Code: Food Science
Methodology Code: Liquid Chromatography
Ion exclusion liquid chromatography (IELC) has been most commonly used to separate relatively short chain aliphatic carboxylic acids. We have optimized an IELC method for the separation of a mixture of aliphatic and aromatic acids representing the hydrolysis of acetylsalicylic acid to its decomposition products salicylic acid and acetic acid. Using a TSK-gel SP-5PW (7.5 mm i.d. x 7.5 cm, 10 μm) column, the mobile phase was optimized to 0.35 mM H2SO4 (pH 3.93) at 1.0 mL/min with an injection volume of 72 μL with UV detection. However, although there was baseline peak resolution, this run time of about 15 min was still considered long. Using a higher flow rate to decrease the run time was not possible due to column operating limits.

This limitation encouraged us to try using a sodium dodecylsulfate (SDS) dynamically modified C18 UHPLC column as we could not easily find such a commercially available strong cation exchange column. We started again with 100% water as a mobile phase at a flow rate of 0.6 mL min⁻¹ (back pressure 8500 psi) and the results were unexpectedly different from those obtained by the standard cation exchange column. The peaks were completely resolved without adding H2SO4 and the injection peak was hardly observable in a 6 min run time. For IELC in the UHPLC mode, the elution order was also different as salicylic acid eluted before acetylsalicylic acid which can be explained by the contribution of the reversed phase retention to the separation mechanism. Analytical figures of merit were similar between the standard IELC and UHPLC modes.

Presently we are trying other sample mixtures such as just aliphatic carboxylic acids and aromatic sulfonates to see if a similar improvement in run time is possible. Variation of the surfactant chain length from C12 to C18 to see how the separation might change with respect to retention factor and peak resolution is also of definite interest.

Keywords: Food Science, HPLC
Application Code: Food Science
Methodology Code: Liquid Chromatography
Commercial market and Internet provide many sources of chemometric software. From the professional application such as those provide by some software editors toward free downloadable toolboxes under various mathematical or statistical environments such as Matlab®, R, Octave or Scilab, many computation tools are available. But, to our best knowledge, very few are usable by neophytes or anyone that are not skilled with chemometrics or statistics. ChemoSoft is the first chemometric software designed for non-chemometricians.

ChemoSoft® allows an easily extraction of the relevant information from data. Its philosophy is different from the usual commercial software. It starts from the goal toward the adequate tool. With ChemoSoft®, user is facing treatment goals and not faced with a multitude of tools that are not always easy to understand. The user has to wonder "I want to see how my data is organized" or "Are there classes or groups in my data? » or «I need for a calibration model for measuring the amount of X in the product A, how doing?" Or simply, the user needs for easily represent spectral or physicochemical data. It guides the user in his goal of processing and displaying by proposing the right tools.

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Keywords: Chemometrics, Data Analysis, Data Mining
Application Code: Food Science
Methodology Code: Chemometrics
As grain products begin to age, they oxidize. One of the products of this oxidation is Hexanal. Hexanal contributes to the green, grassy odor in food and can ruin a good morning when breakfast takes on an unpleasant taste. The amount of Hexanal in foods is one indicator of the expiration date. Since Hexanal can be detected through static headspace and SPME sampling, this study will examine Hexanal detection using both sampling techniques using a new automated sampling system that can perform sample preparation on top of the GC. This examination will also determine how Hexanal levels increase as cereal is allowed to age in an unsealed container.
Patulin is a mycotoxin produced by several Penicillium, Aspergillus and Byssochlamys mold species and is commonly found on apples. Apples which have been damaged or bruised prior to processing are more susceptible to contamination by patulin-producing molds. When patulin contaminated apples are processed into juice, high levels of patulin are possible. The thermal stability of patulin prevents its decomposition during pasteurization.

The U.S. FDA has set a maximum residue level (MRL) of 50 ug/kg of patulin in apple juice and apple juice concentrates. Other countries including China, Japan and the EU have also set the maximum contamination of patulin to 50 ug/kg in apple juice products. The EU also has lower limits for patulin in solid apple products (25 ug/kg), such as apple puree, and products designed for infants and young children (10 ug/kg).

To protect both the producer and consumer, accurate testing is required to prevent the contamination of apple juice with patulin. To improve the selectivity and reduce limits of quantification, mass detection is desirable in the analysis of patulin. In order to offer laboratories the opportunity to capture the benefits of mass detection without the challenges associated with the adoption of mass spectrometers, the ACQUITY QDa Detector has been developed.

Single ion recording (SIR) was used to monitor patulin and RADAR technology allowed for the simultaneously acquisition of full spectrum data. Patulin was successfully detected in apple juice and mass detection with SIR provided high selectivity for this analysis. Patulin was detected down to spiked concentrations of 1 ug/L. The lowest spiked concentration that resulted in a signal-to-noise (S/N) ratio above 10 (using the peak-to-peak method) was 5 ug/L. This level is ten times lower than required by the EU and FDA regulations for apple juice and half of the strictest EU level for baby food.

Keywords: Beverage, Detector, Instrumentation, Liquid Chromatography/Mass Spectroscopy
Application Code: Food Science
Methodology Code: Liquid Chromatography/Mass Spectrometry
Various traditional edible products which have useful ingredients cannot be used only because of their bitter taste or texture. Oil, olive oil, ice cream, etc. have shape retention properties by mixing with a few % of microfilaments of starch cotton. These hold the shape even at 200°C, where these become baked brown-black. When these are eaten, these rapidly liquefy by salivary amylase. These have convenient easy-to-use hardness at any temperatures from minus 20 to plus 200°C. This has no harm because of no trans-fatty acid, saturated fat, wax etc. USA patent 2011-125301.

To mask bitterness of edible substances containing pharmaceuticals, food, or drink, whether a fine powder or a whole fruit, safely, simply, with anti-bacterial and anti-degenerative properties over the long term even at room temperature and in a hydrated state, without altering the composition, to be absorbed by the stomach. Solution: To use an edible flocculating agent in use from early times. Nutritious bitter olive polyphenols become delicious and beautiful, i.e. olive red wine, vinegar, nuts, alcoholic dishes, sweet, etc. without NaOH or salt treatment. Polyphenol content 40mg/dl. They are delicious in mouth but return to original in stomach. The reactive part of bitter, which will bind to taste bud receptor, is coagulated with saturated Ca(OH)2 & CO2, and covered. After they are eaten, gastric acid immediately dissolves the cover. Because of the bitter, olive produces massive wastes, e.g., oil cake, nuts which are eggs of whole olive, NaOH& saline pickling which destroy nutritions. This method can be used for bitter melon( Momordica charantia), tea, bile, Rhei Rhizoma(bitterest medicine), etc. Only the bitters are masked. The other all tastes, such as deliciousness etc. do not change. The masked Rhei showed the same medical effect as original. Any kind of calcium can be used, i.e. any wastes of themselves. To eat nutritious olive have been impossible until now.

Patent pending WO2012/036080A1

Keywords: Chemical, Drugs, Environmental, Food Science
Application Code: Food Science
Methodology Code: Chemical Methods
In the production process in starch industry, the protein content, calculated through the Nitrogen determination, is periodically monitored and tested for quality control. Considering the fact that the starch is used also in the preparation of animal feeds, the determination of N/Protein becomes critical.

Therefore it is very important to have a method which permits the analysis of N/Protein with an excellent reproducibility. For this reason the FLASH 4000 Nitrogen/Protein Analyzer, based on the dynamic flash combustion of the sample, fits the bill as it satisfies all the requirements of modern laboratories such as stability, accuracy, day-by-day reproducibility and high sample throughput. This alternative to the classical Kjeldahl, based on Dumas (combustion) method, has been developed and approved by different associations. This paper presents data on Nitrogen/Protein determination of different starch samples in a large range of concentration (150 – 2000 ppm Nitrogen), obtained with the analyzer using large sample weight to demonstrate the validity of the method without matrix effect. Some data compared to the results obtained by the Kjeldahl method demonstrates the validity of the system.
Improvements in instrument speed, selectivity, and sensitivity have significantly lifted some of the burden associated with analytical testing; however, as the instrumentation evolved, so have the sample preparation options, enabling laboratories to adopt methodologies that offer advantages in terms of time savings, solvent usage, and overall cost. The QuEChERS approach is an example of this evolution in sample preparation. QuEChERS as a food sample preparation method was quickly adopted by laboratories trying to save time and money while still effectively cleaning up increasingly complex samples. However, while optimizing sample preparation conditions can be as simple as exploring salt and sorbent variations, it can also include modifications that account for the sample pH, texture, and matrix complexity. This work presents several applications where modifying a standard QuEChERS method to suit the sample type was required. Pesticides in animal tissue and juice concentrate, component analysis in botanicals, and pet food additives, among other challenging applications, will demonstrate how a comprehensive but simple method development approach can deliver superior method performance.

Keywords: Food Science, Gas Chromatography/Mass Spectrometry, Liquid Chromatography/Mass Spectroscopy,
Application Code: Food Science
Methodology Code: Sampling and Sample Preparation
Very Large Range Pesticide Screening in Food Using GC Triple Quadrupole MS

Greater than before ease of access to high selectivity GC-MS is allowing more rapid and generic sample preparation in pesticide testing, permitting consolidation of multiple analyte lists and matrices into one analytical workflow. While GC-MS/MS is well suited to multi-residue analysis in a diverse range of matrices, an increased number of targeted compounds also amplifies the complexity of method optimization and analytical performance may become problematic. Furthermore, there is sometimes a desire to look beyond targeted lists for other potentially harmful food contaminants. Presented is the use of smart instrument control and data processing software applied to GC-MS/MS analysis of >600 pesticides in matrix to mitigate analytical performance degradation through MS duty cycle optimization. Also discussed is combining this optimized targeted quantitation with general unknown analysis through fullscan/MRM.

Keywords: Food Science, Gas Chromatography/Mass Spectrometry, High Throughput Chemical Analysis, Pesticide
Application Code: Food Science
Methodology Code: Gas Chromatography/Mass Spectrometry
Introduction

Fraudulent and adulterated food products are a multibillion dollar a year business. Not only are consumers not getting what they pay for, but in some cases the fraudulent products have led to deaths due to allergens or poisons being present in the product. Truffle oil is one of the top 10 foods that are found to contain adulterants or outright fraudulent.

Methods

Analysis were conducted using a Thermal Desorption system coupled to a GC/MS operating in El ionization mode. Direct Thermal Extraction was used for the Truffle oil. A small sample (10-50 mg) was placed inside a pre-conditioned thermal desorption tube and desorbed directly into the GC injection port, the GC column passed through a Cryotrap cooled with Liquid CO2 to reduce band broadening during the desorption process.

Results

The truffle oil tested contained many adulterants with 2,4-dithiapentane, a synthetic based petroleum flavoring agent, being one of the main components.

Novel Aspects

TD/GC/MS is capable of monitoring for contaminants and adulterants in food products without the need for solvent extraction.

Funding was provided by Scientific Instrument Services

Keywords: Flavor/Essential Oil, Food Science, GC-MS, Thermal Desorption
Application Code: Food Science
Methodology Code: Gas Chromatography/Mass Spectrometry
Olive oil can become contaminated with polynuclear aromatic hydrocarbons (PAHs) through exposure of the olives to pollution in the environment. Concern over exposure to these compounds has resulted in European Union Commission Regulation No 835/2011. This regulation sets a maximum limit for PAHs in edible oils of 2 ng/g benzo[a]pyrene alone, and 10 ng/g total for the sum of benzo[a]pyrene plus three additional PAHs. Low level PAH analysis is commonly done by either GC-MS or HPLC-FLD. Oily/fatty samples present an analytical challenge due to the heavy matrix effects often encountered. In the case of GC-MS, fatty matrix can cause contamination of the GC inlet, column and detector. In the case of HPLC, matrix can build up on the column, resulting in loss of chromatographic efficiency and/or an increase in system backpressure. Various cleanup techniques exist for fatty samples, and some can be time consuming and expensive. In this work, a new SPE cartridge containing two different sorbent layers was evaluated in the simultaneous extraction and cleanup of PAHs from olive oil. The layers consist of Florisil and a mix of Z-Sep/C18. Olive oil sample was loaded directly onto the SPE cartridge, followed by elution of the PAHs with acetonitrile while fatty matrix remained bound to the sorbents. The resulting extract was concentrated, and analyzed by both GC-MS and HPLC-FLD. The dual-layer SPE cartridge was evaluated with olive oil samples spiked with light and heavy PAHs, and found to yield recoveries of >70% and % RSD values <15% for most compounds.

Keywords: GC-MS, HPLC, PAH, Solid Phase Extraction
Application Code: Food Science
Methodology Code: Sampling and Sample Preparation
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. The rise in popularity of this technique and the increase in sample testing have driven the need for automation of the QuEChERS method to increase productivity and throughput. The AutoMate-Q40 streamlines the QuEChERS method from adding Acetonitrile (ACN) and buffering salts, shaking, mixing, centrifugating the sample, transferring to a dispersive solid phase extraction (d-SPE) tube, measuring and delivering the extract. The aim of this project is to validate the performance of AutoMate-Q40 by monitoring various chemical residues in different matrices. The target residues will be determined by Liquid Chromatography tandem mass spectrometry.
A Highly Sensitive, Real Time LSPR Sensor

Optical sensors based on localized surface plasmon resonance (LSPR) exploit the dependence of the resonance frequency on local refractive index. The shift is determined by analyzing, often with complex software, the position of the LSPR peak before and after analyte addition. Here, we present a new approach to measuring the shift in the LSPR spectra by monitoring the extinction of a plasmonic structure at two different wavelengths, one on the red side of the peak and the other on the blue side. When the LSPR peak shifts in either direction, the extinction at one wavelength will increase while the other will decrease and we look at the differential of these two signals for our measurements.

The uniqueness of our approach comes not only from real time differential measurements, but also from using an LSPR substrate with an exceptionally sharp resonance originating from plasmon coupling in a self-assembled 2D array of closely spaced silver nanoparticles (AgNP). A typical array of 100nm AgNP exhibits a FWHM as narrow as 10 nm, contrary to 100+ nm seen in more common SPR systems. As a result of this sharp peak, small changes in its position result in large changes in the differential measurement. Differential measurements with sharp peaks has allowed us to detect changes, in bulk refractive index of solutions, down to 1.4E-5 and was measured in 1 second with a signal to noise ratio of 4. This approach provides advantages such as increased sensitivity, reduced detection limit, and real time analysis.

Keywords: Detector, Nanotechnology, Sensors, Spectroscopy
Application Code: General Interest
Methodology Code: Sensors
Recently, wireless sensing of key analytes has been explored with photosensitive molecules activated by UV light, which can be damaging. Here, we use a visible light responsive meta-stable merocyanine photoacid polymer for photosensing membranes. The lipophilic photoacid polymer synthesized has been fully characterized via UV-Vis spectroscopy, NMR and Gel Permeation Chromatography. The photoacid unit of the polymer undergoes proton dissociation under visible light irradiation with long-lived changes in pH. This change is completely reversible and follows 1st order kinetics. The observed half-life of the proton dissociated state of the photoacid in the sensing membrane is ~2 hours. This time is suitable for proton exchange with analyte cations, thus providing a sensing mechanism. Membranes based on polyvinyl chloride with plasticizer, lipophilic photoacid polymer and a sodium ionophore have been shown to actively exchange protons with sodium ions under visible light (470 nm) irradiation, and the exchange can be followed by UV-Vis spectroscopy. The membrane shows no response to sodium in the OFF state (dark). The limit of detection of the sensor is $1.0 \times 10^{-5}$ M and each sensing membrane can be used over multiple cycles with no observable fatigue. Further work is in progress to improve limits of detection and response times and to extend the sensing mechanism to different cations. Thus, these results are bringing us closer to novel types of cost effective wireless sensors. The authors acknowledge the College of Sciences and the Department of Chemistry at the University of Central Florida for financial support of this research.

**Keywords:** Absorption, Analysis, PAH, Sensors

**Application Code:** Bioanalytical

**Methodology Code:** Sensors
To choose an appropriate material to seal samples of organic solvents, a measurement device was built to monitor the concentration on each side of the septum. The time until a rapid rise of concentration on the clean side of the membrane is measured and is used to qualify a membrane material. To fulfill the objectives of this application, a sensing device based on a photo ionization detector was developed. The PID has an ionization potential of 10.6 eV, so most organic compounds can be detected. The PIDs analog output signal is amplified and converted to a digital dataset for further signal handling by an embedded microprocessor. A stored calibration curve allows a reconstruction of the concentration according to the response factor of the substance. The measured value is stored and displayed. The measurement device detects organic compound as low as 50 ppb (response factor: 1). The data can be transferred by a USB interface to a PC. In this application, the device samples data every second. A gas sample is generated by evaporation of the substances liquid phase in a defined temperature and flow condition. Compressed air is used as carrier gas. Then the permeation rate through the membrane is monitored over several days. The design of the measurement device and the experimental setup will be presented. Measurements for different membrane materials will be shown.
**Abstract Text**

Single-walled carbon nanotubes (SWCNTs) are functionalized with a trifunctional selector to yield a highly robust and sensitive chemiresistors to selectively detect cyclohexanone vapor, a target analyte for explosive detection. The trifunctional selector has three important properties: it noncovalently functionalizes SWCNTs with cofacial interactions, it binds to cyclohexanone via hydrogen bond, and it improves the overall robustness of SWCNT-based chemiresistors. We have proven the mechanism behind the molecular recognition of our sensors by methylating the acidic protons of the thiourea receptor while observing the sensor’s corresponding conductive change upon exposure to cyclohexanone vapor. We also determined the trifunctional selector’s alkoxy silane group provides remarkable robustness for the sensor to be resistant towards humidity, heat, and mechanical damage (e.g. sonication). Overall, our sensors produced reversible and reproducible responses in less than 30 s to 10 ppm of cyclohexanone and displayed an average theoretical limit of detection (LOD) of 5 ppm.

**Keywords:** Materials Science, Nanotechnology, Sensors, Volatile Organic Compounds

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Sensors
It is necessary to determine carbonate ion quantitatively in clinical analysis, since carbonate ion plays an important role in the living body. Ion-selective electrodes are useful for determination of a certain ion in the presence of other ions. However, conventional ion-sensing membranes, plasticized poly(vinyl chloride) membranes, may not be proper for biological samples without pretreatment, because they are not biocompatible. In this study, we adopted sol-gel glass, which is biocompatible, as a membrane material and sol-gel-derived membranes Modified Chemically by ionophores were used. The molecular-tweezer type ionophore bearing two trifluoroacetophenone groups was used, where a triethoxysilyl group was introduced. The mixture of tetraethoxysilane (TEOS) and diethoxydimethylsilane (DEDMS) containing the synthesized ionophore and a quaternary ammonium salt bearing trialkoxysilyl group was heated to about 80ºC, for 1 hour, and an obtained viscous liquid was coated to a gate of ISFET. After heating for 1 day, ion-sensing membranes were obtained. The molecular ratios of DEDMS to TEOS were changed over a range from 3.2 to 4.0. The higher the ratio of DEDMS were, the softer the membranes were. The obtained membranes responded to carbonate ions, and the slopes of EMF responses became larger when the softer membranes were used. The membrane containing 20 wt% of the ionophore and 60 mol% of the ionic site relative to the ionophore (TEOS : DEDMS = 1 : 3.8) showed a Nernstian response. The selectivity coefficients of carbonate ion with respect to other anions were smaller than those of a sol-gel-derived-membrane containing only the ionic site.
Terahertz time domain spectroscopy (THz-TDS) has the ability to probe the crystallinity of several materials, due to the interaction of THz radiation with optical phonons in crystal lattices. In this work, THz-TDS has been used to quantify the degree of crystallinity of microcrystalline cellulose (MCC) samples. The THz spectra of cellulose presented features which could be directly correlated with the crystallinity index (CI) obtained by means of the well established powder x-ray diffraction (PXRD) technique. Both univariated and partial least squares (PLS) calibrations were carried out to correlate the THz spectra with CI, and yielded results comparable to PXRD and a root mean square error of cross validation of 0.035 for the CI.

**Keywords:** Chemometrics, Materials Science, Molecular Spectroscopy, Spectrophotometry

**Application Code:** General Interest

**Methodology Code:** Vibrational Spectroscopy
Number of bioanalytical systems and sensors devoted for the determination of antibodies, which are indicating some infections, were developed. Such sensors are based on various methods including: electrochemical impedance spectroscopy [1]; fluorescence and fluorescence quenching [2]; application of potentiodynamic methods, which allow direct detection of antibody-antigen complex formation [3]; application of surface plasmon resonance (SPR) based methodologies [4]; usage of combined SPR/Electrochemistry/Electroluminiscence based techniques [5]; adjustment of ellipsometry based techniques [6,7,8], application of SERS based analysis [9]; and adaptation of micromechanical capacitors (cMUTs) [10].

In this study recent advances in application of SPR and Ellipsometry based techniques in the design of immunosensors were presented. Comparison of analytical parameters of SPR and Ellipsometry based techniques was made and advantages and disadvantages of both techniques were discussed. Application of chemically modified antibodies was outlined as very promising technique, which increases sensitivity and selectivity of evaluated immunosensor. Attempts to immobilize antibodies in oriented way and ellipsometric evaluation of gold substrates modified by oriented and not-oriented antibodies were evaluated.

Acknowledgement
This project was financially supported by Lithuanian Scientific Council.

**Abstract Text**

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**Acknowledgement**

This project was financially supported by Lithuanian Scientific Council.
Abstract Text
Homocysteine has been recognized as an important risk factor for vascular disease and Alzheimer's disease. A novel redox-based fluorescent probe has been developed for homocysteine detection. Based on redox reaction with the thiol group, the probe selectively responds to homocysteine over other biologically abundant thiols such as cysteine and glutathione, leading to large fluorescence intensity changes. In addition, quantitative analysis of homocysteine can be achieved in the linear range of 0-100 µM in phosphate buffer and diluted deproteinized plasma.

Keywords: Amino Acids, Detection, Fluorescence, Sensors
Application Code: General Interest
Methodology Code: Sensors
There’s a growing interest in detecting nitrite because of its important role in many areas\(^1\). Ionophore-based polymeric membrane potentiometric and optical sensors with adequate nitrite selectivity have potential applications for nitrite detection in biological and environmental samples. A number of ionophores with nitrite binding affinity have been utilized to fabricate nitrite-selective membrane electrodes, but few have been used to develop nitrite-selective optodes. Our group has previously examined Rh(III) porphyrins\(^2\) and Co(III) corroles as ionophores to detect nitrite potentiometrically, and both yield membrane electrodes exhibiting Nernstian and selective response to nitrite. To investigate these same ionophores for the development of nitrite-selective optodes, both neutral chromoionophores and charged chromoionophores with different pKa values are employed along with the metal ion-ligand ionophores for preparing the optical sensing films. In general, optical sensors with these two types of ionophores show good selectivity to nitrite over other anions, which is consistent with potentiometric results. For polymeric films formulated with Co(III) corrole and neutral chromoionophores, it is found that chromoionophores with higher pKa values yield optical sensors that have more sensitive response to nitrite. For films prepared with the Rh(III) porphyrin species, operation of the ionophore via a charged carrier mechanism yields better selectivity towards nitrite over other anions than when films are prepared to employ the ionophore as a neutral carrier. Detailed analytical parameters and performance of these different optical nitrite sensing films will be presented. We acknowledge the National Institutes of Health (EB-000784) for supporting this research. (1)Benjamin, N.; Odriscoll, F.; Dougall, H.; Duncan, C.; Smith, L.; Golden, M.; McKenzie, H. Nature 1994, 368, 502. (2)Pietrzak, M.; Meyerhoff, M. E. Anal. Chem. 2009, 81, 3637.

**Keywords:** Ion Selective Electrodes, Sensors, Spectroscopy, UV-VIS Absorbance/Luminescence

**Application Code:** General Interest

**Methodology Code:** Sensors
Self-assembled noble metal nanoparticles have attracted many researchers since these nanostructure-depending unique plasmonic properties are available for sensing with surface-enhanced Raman scattering (SERS). We demonstrated the casting method in convective flow under controlled liquid-solid interfaces for the fabrication of gold nanoparticles three dimensional nanostructures (Au3D). A PDMS sheet with a 6 mm through hole was attached on the uv-cleaned slide glass, where hydrophobic PDMS wall ($\text{S} = 112.0$) of the 6 mm well made a circumference of “coffee-ring” so as to pin a drop of the PDMS AuNP (citrate capped, 20 nm) and PSL (sulfate type, 600 nm) mix dispersion. Since the hydrophobic surface of PDMS also made a convex meniscus of the drop around which surface/volume ratio was larger than other part of the drop, evaporation of water was accelerated around the circumference. In addition, all solids and particles had negatively charged. Their coulomb repulsion promoted a convective transport of particles toward evaporation front. As a result, after washing with dichloromethane to remove PSL from the thickly stacked self-assembly on the edge of “coffee-ring”, sterically-bulky nanostructures with enough thickness was obtained. The geometric parameters confirmed us that the characteristic length of the obtained Au3D was enough large corresponded to a spot size of incidental laser, not only horizontally but vertically. In SERS measurements, Raman scattering by irradiation of 785 nm (3 mW) laser for 1 second on the Au3D immersed in 4,4’-bipyridine (4bpy) aqueous solution were collected. Excellent SERS spectra appeared within 10 min after immersion of 100 pM 4bpy (red line and inset) and 100 nM (blue line), while any peak was not observed in blank measurement (dotted line) of Au3D. Three or four 4bpy molecules were reasonably considered to exist in the observed volume of 4bpy 100 pM solution.

**Keywords:** Nanotechnology, Sensors, Surface Enhanced Raman, Ultratrace Analysis

**Application Code:** General Interest

**Methodology Code:** Sensors
Disposable Microelectrode Ensembles Fabricated with Toner Masks for Hydrogen Peroxide Determination

The fabrication of disposable electrochemical sensors with certain specific features (possibility of large scale production, application "in loco", reproducibility of the electrode area and low cost) is one of the growing areas in analytical chemistry. This work describes the fabrication and application of a disposable microelectrode ensemble (DME) modified with a toner mask. The DME was fabricated by deposition of two toner mask layers and its design was drawn using a graphical software (CorelDraw X5). Firstly, the disc area (2 mm diameter) was delimited with toner mask. Then, a second layer was printed with 90% black toner mask. The last step was the heat-transfer of the toner mask to a copper surface in a procedure with optimized parameters of 120°C and 10 min using a thermal press equipment. The proposed electrochemical sensor was applied for the amperometric determination of hydrogen peroxide in a real sample (oral antiseptic) at -0.2 V. A linear relationship between the peak current and concentration of hydrogen peroxide was obtained in the range 0.02 to 2.6 mmol L\textsuperscript{-1} with detection and quantification limits of 0.2 (S/N=10) and 0.6 (S/N=10) [micro]mol L\textsuperscript{-1}, respectively. The value found with the proposed sensor was 0.48 ± 0.02 mol L\textsuperscript{-1} and the result was in agreement with those obtained by using a recommended procedure and with the value labeled by the manufacturer. The good results obtained with the DME envisage the use of this proof of concept for fabrication of DME with other electrodic surfaces such as gold or carbon.

Keywords: Analysis, Array Detectors, Detection, Sensors
Application Code: General Interest
Methodology Code: Sensors
Iron is an abundant element in the environment which plays an important role in environmental, industrial, human and biological systems, and as such, is of key interest for analytical studies. Amperometric sensors for Fe(III) present several advantages based on low detection limits, large dynamic concentration range, good selectivity, rapid response time and inherent miniaturization and portability. However, these features depend on the use of mediators capable of recognizing the analyte in complex samples. In this work, the electrocatalytic reduction of Fe(III) was investigated using a carbon fiber microelectrode (CFM) modified with a ruthenium oxide hexacyanoferrate (RuOHCF). At surfaces containing this chemical modifier, Fe(III) is electrocatalytically reduced at 0.0 V without interference from Fe(II), hence allowing the speciation of Fe(III). A linear relationship between the cathodic current and concentration of Fe(III) was obtained by amperometry in the range 10–210 [micro]mol L[sup]-1[/sup] with detection and quantification limits of 0.22 (S/N=3) and 0.74 (S/N=10) [micro]mol L[sup]-1[/sup], respectively. This sensor will be used for analysis in industrial waste samples.

**Keywords:** Chemically Modified Electrodes, Environmental/Water, Sensors, Speciation

**Application Code:** Environmental

**Methodology Code:** Sensors
Surface Plasmon Resonance Sensing is well-known as highly sensitive mass transducer. It is able to detect mass change at the interface in picogram order. Common SPR employs the Kretchman’s configuration and flow injection. However, the stabilization of the flow injection is a challenge on the commercialization, because the solution flow causes many troubles such as liquid spill, stacking, bubble, and so on. Here, we will propose the total SPR sensing system that requires no flow injection. In our proposed assay protocol, the immunoassay test chip was examined in the bulk sample solution. And then, the immunoassay test chip was evaluated by SPR reader system based on Otto-Configuration. The surface Plasmon wave could be coupled with the Evanscence field at the boundary between Prism and Au sensor chip. This configuration does not need flow injection. Therefore, “a low-failure risk” could be achieved. In addition, the signal amplification using Localized Surface Plasmon Resonance (LSPR) will be discussed here. LSPR can instantly enhance the SPR response, because the surface electromagnetic field could be amplified by localized coupling. Although the SPR signal of Otto-configuration is low in ordinary immunosensor chip, it could be remarkably enhanced by LSPR effect.
Gold nanoparticles (AuNps) are very interesting materials for sensor application especially owing to their plasmonic properties. Surface plasmon resonance (SPR) combined with Raman spectroscopy provides a very sensitive analytical tool, making possible to detect concentration bellow 10-9 mol dm-3 or parts per billion. However, at lower concentrations the stochastic behavior of the hotspots becomes pronounced, compromising the reproducibility and consequently the analytical application. We found out that the association of superparamagnetic iron oxide nanoparticles (SPIONs) to plasmonic nanoparticles is a convenient combination because allows magnetically induced concentration of the AuNPs, leading to the formation and concentration of hotspots. In this work we demonstrate a simple way to improve up to sixty folds the signal-to-noise ratio of conventional SERS probe, improving the detection limit by about the same magnitude.

AuNps and SPIONs were prepared according to the Turkevich [1] and Park et al. [2], respectively. AuNps suspension was interacted with phenanthroline in concentrations ranging from 10-9 to 10-11 mol dm-3, and Raman spectra were recorded exciting at 785 nm. SPIONs were added to the AuNps suspension and the composite nanomaterial was magnetically concentrated on a quartz cuvette wall using a small FeNdB magnet, and Raman spectra were recorded. This simple procedure allowed us record spectra with enhanced peak intensities in a reproducible way, whose intensity was proportional to the concentration of that molecule in solution. This result contrasts with those obtained with the traditional and laborious Au@SPION core-shell and gold nanoparticles decorated SPIONs described in the literature.


Keywords: Nanotechnology, Raman, Sensors
Application Code: Nanotechnology
Methodology Code: Sensors
The measurement of pH in solutions containing hydrofluoric acid is important for some industrial applications, in particular for controlling the operation of wastewater treatment equipment. There are commercially available hydrofluoric acid-resistant pH sensors based on specially formulated silicate glasses or metal oxides, but their measurable pH range is limited to pH values higher than 2 and the hydrofluoric acid concentration of the sample has to be less than 1000 mg/L.

We have found that a liquid-membrane ion electrode containing a hydrogen ion-selective neutral carrier is resistant to hydrofluoric acid. Based on the optimization of the membrane composition, a poly(vinyl chloride) membrane electrode has been developed using octadecyl isonicotinate (ETH1778) as a neutral carrier, tetrakis[3,5-bis(trifluoromethyl)phenyl]borate as an ionic additive and 2-nitrophenyl octyl ether as a plasticizer. The electrode shows a pH response in a working range of pH 0.5-5 and can be used in solutions containing up to 10,000 mg/L of hydrofluoric acid. The lifetime of the electrode is at least 6 months for continuous pH measurements.

The main advantages of Surface Enhanced Raman Spectroscopy (SERS) are specific and fast measurements. Strongly increased Raman scattering signal is generated by local field enhancements near metallic nanostructures and the internal modes of the reporter molecule can be used as diagnosis signals. However, appropriate placement of the reporter molecule on the metal nanoparticle surface and aggregation are big challenge. Several approaches have been suggested for this purpose and multipurpose functionalized hybrid nanoparticles are very promising for the detection of trace amounts of analyte. In this work, new hybrid magnetic nanoparticles which are suitable for biomolecules immobilization were synthesized in order to develop a homogeneous SERS assay platforms for the detection of pathogenic microorganisms and toxins. Rapid, easy and room temperature synthesis of magnetic nanoparticles and subsequent modification with chitosan polymer provide magnetic separation and SERS applications. The optimization strategies for avoiding aggregation and the analytical performance of the SERS-based assays will be presented.
The interplay of biological factors is seldom investigated simultaneously, yet to obtain an understanding of the interactions involved, multi-analyte monitoring with high temporal resolution is required. Multiple peptides released from islets of Langerhans, such as insulin, glucagon, and islet amyloid polypeptide, help regulate blood glucose. The peptide levels are known to oscillate in vivo with periods ranging from minutes to hours. While the causes of these oscillations are unknown, these complex secretion patterns have been shown to have a positive effect on peripheral tissue function and are perturbed in diabetes. In this talk, various separation and detection methods we have developed to simultaneously measure multiple peptides secreted from islets of Langerhans with high temporal resolution will be described. The methods are being applied to the examination of the dynamic behavior of both individual and groups of islets under in vivo-like conditions. In addition, optimization methods for capillary and microfluidic electrophoretic separations and post-separation sample handling will be described.

Keywords: Bioanalytical, Lab-on-a-Chip/Microfluidics, Separation Sciences
Application Code: Bioanalytical
Methodology Code: Separation Sciences
Petroleum crude oil presents the world's most complex organic mixtures. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) offers a peak capacity greater than 1,000,000, providing nonpareil resolution and identification of >100,000 elemental compositions from a single mass spectrum. However, exact mass measurement alone does not distinguish isomers, and the finite number of trapped ions limits dynamic range. GCxGC (with time-of-flight MS or element-specific detection) can resolve isomers for molecules containing up to ~35 carbons, and LC performed with a series of eluents of increasing polarity can increase dynamic range and separate different functional groups (e.g., alcohol vs. ketone). The combination of these techniques provides unprecedented compositional and structural detail for crude oil and its products. Work supported by NSF Division of Materials Research through DMR-11-57490, BP/The Gulf of Mexico Research Initiative to the Deep-C Consortium, the Florida State University Future Fuels Institute, and the State of Florida.

Keywords: Fuels\Energy\Petrochemical, HPLC, Mass Spectrometry, PAH
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Mass Spectrometry
We have developed an all-electric approach to the measurement of endogenous species in brain tissue cultures. Electroosmotic flow is induced in an organotypic hippocampal slice culture. The electric field carries fluid to a microfluidic chip where thiols react with a fluorogenic reagent, solutes are separated by electrophoresis and quantitated by laser-induced fluorescence. Exogenous compounds can be passed through the tissue as well. The measurement then provides information about the metabolism of the perfused species. We have applied this measurement to a determination of CoA metabolism. The tight integration of the living tissue and the instrument is important in measurements of reactive species such as thiols.

Keywords: Amino Acids, Capillary Electrophoresis, Fluorescence, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Reactive nitrogen species such as nitric oxide and peroxynitrite participate in oxidative stress and nitration/nitrosylation in vivo. These species have also been implicated in several cardiovascular and neurodegenerative diseases. The short lifetime of these molecules makes them difficult to detect, often requiring indirect methods of analysis. Microchip electrophoresis (ME) offers fast and efficient separations, allowing these species to be separated and detected before they degrade significantly. In addition, a buffer with high pH stabilizes peroxynitrite as its anion. In this project, ME with amperometric detection was utilized to separate and detect reactive oxygen and nitrogen species as well as endogenous antioxidants from stimulated RAW 264.7 murine macrophage cells. A separation of tyrosine, glutathione, hydrogen peroxide, peroxynitrite, and nitrite was developed using reverse polarity. This separation was then applied to the analysis of RAW 264.7 cell lysates. The macrophages were first stimulated with lipopolysaccharide and interferon gamma to generate nitric oxide. A peak appeared in the electropherogram that comigrated with the nitric oxide standard and decayed over time as expected. In a separate experiment, cells were also exposed to polymyristol acetate to generate peroxynitrite. Several dual electrode configurations were developed for on-the-fly verification of peak identity based on voltammetry. This is especially important for labile species. The ultimate goal of this project is to couple this separation and detection method with on-chip, single cell lysis to quantify these species and gain a better understanding of their role in oxidative stress and nitration.

Authors acknowledge funding from NIH NINDS, KU SELF Foundation and the Adams Institute.

Keywords: Electrochemistry, Electrophoresis, Lab-on-a-Chip/Microfluidics

Application Code: Bioanalytical

Methodology Code: Microfluidics/Lab-on-a-Chip
Most proteomics studies employ LC-ESI-MS/MS analysis of peptides. We are investigating capillary electrophoresis (CE)-ESI-MS/MS as an alternative technology for bottom-up proteomics. We first developed a rugged and sensitive CE-ESI interface based on electrokinetically-pumped sheath-flow (1). This interface operates in the nanospray domain, produces low-attomole detection limits for capillary electrophoresis separation of peptides, and offers great flexibility in separation buffer conditions (1-2). We then analyzed the secreted protein fraction of M. marinum (3). We pre-fractionated the secretome to produce simpler samples that were better suited to the separation performance of capillary zone electrophoresis (CZE). The results of the analysis of 12 fractions were compared with conventional UPLC analysis of the unfractionated sample. CZE produced slightly more protein identifications in a slightly shorter time period than UPLC. 140 protein groups were identified by CZE-ESI-MS/MS in three hours from this sample. We have recently improved the system. In the single-shot analysis of the E. coli proteome, we identified >1,300 peptides and >300 protein groups in a 50-min CZE separation (4). We have employed this separation system to analyze seven fractions from the E. coli proteome (5). This system produced 23,706 peptide spectra matches, 4,902 peptide IDs, and 871 protein group IDs in 350 min analysis time. In an alternative separations scheme, we employed capillary isoelectric focusing for the analysis of differential protein expression in PC12 cells undergoing differentiation following treatment with nerve growth factor (6); we identified 835 protein groups and produced 2,329 unique peptides IDs.

5. Yan et al. Proteomics in press

Keywords: Capillary Electrophoresis, Mass Spectrometry, Proteomics
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Partial least squares, PLS, has been a workhorse in chemometrics for more than three decades to relate two blocks of data. Orthogonal projection to latent structures (OPLS), is a recent modification of PLS that has demonstrated great success.

The driving force behind OPLS was that PLS model interpretation was shown to be confounded by different systematic variations in the data. The OPLS method separates these different systematic variation in X into two parts, one that is correlated (Predictive) to Y and one that is uncorrelated (Orthogonal) to Y. This has consistently shown to facilitate model interpretation and make it more straightforward.

Because of the benefits demonstrated, OPLS has now been developed into a suite of different variants to fit different purposes. E.g. OPLS-DA for discriminant analysis, K-OPLS for non-linear modeling, O2PLS and OnPLS for multi-block data integration.

I will present several examples and applications.

Keywords: Bioinformatics, Biotechnology, Chemometrics, Data Mining
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Chemometrics
In chemometrics, two very different classes of mathematical tools, self-modeling curve resolution and hard-modeling (first-principles) methods, have been developed to resolve pure component concentration profiles and spectra from mixture spectra recorded over time during dynamic processes in application areas from overlapped chromatographic peaks to industrial batch processes. This paper presents advantages and disadvantages of each approach via examples, and two novel applications for modeling dissolution, reaction and crystallization process in one comprehensive first-principles model.

In self-modeling curve resolution (SMCR) methods, realistic constraints such as non-negativity of concentration profiles or equality constraints for known pure component spectra are imposed to produce solutions that obey the constraints and Beer's law. In many cases, SMCR may be the only method available for resolving the pure component profiles; however, it is widely appreciated that in most circumstances, SMCR techniques do not produce unique mathematical solutions, rather a family of feasible solutions that obey boundaries imposed by the constraints. In this presentation, SMCR with a method for computing the range of feasible solutions is illustrated. An algorithm for SMCR that yields improved results by use of soft constraints with penalty functions is also described.

Methods of fitting first-principles multivariate kinetic models are powerful alternatives to SMCR. Such modeling methods do not suffer from ambiguities in the resulting solutions. In process monitoring and control applications, numerical fitting of comprehensive kinetic models use dynamic information to estimate reaction rates, chemical equilibria, process states, end-points, deviations from optimal performance and can provide mechanistic information for process adjustment and optimization.

Keywords:  
Chemometrics, Process Analytical Chemistry, Process Monitoring, Spectroscopy

Application Code:  
Process Analytical Chemistry

Methodology Code:  
Chemometrics
There is an increasing need to develop analytical methods to assess protein structure and conformational changes in real or near-real time. Protein conformational changes are associated with a number of diseases and protein interactions with small molecular compounds can also lead to subtle but important structural changes. Structurally sensitive, optical methods derived from UV absorption, infrared and Raman spectrosopies are ideal candidates for addressing this need. However, each technique has its own strengths and weaknesses. Circular dichroism (CD), the gold standard in protein secondary structure analysis provides little more than a rough estimate of secondary structure content, with [beta]-sheet and disordered conformations being poorly approximated. While changes in secondary structure can be easily identified by CD, assignment of those changes is much more ambiguous. Deep-UV resonance Raman (dUVRR) spectroscopy is an emerging technique in the field of protein secondary structure analysis. Though conformational changes are more easily assigned by dUVRR, prediction of secondary structure content by dUVRR suffers the same limitations as CD with [alpha]-helical and disordered structure being poorly approximated. In order to take advantage of the unique selectivity's of each technique, chemometric methods are needed to combine information derived from multiple spectroscopic techniques. While combining two types of spectral data is the most straight-forward data fusion strategy, unequal spectral intensities, noise levels and spectral overlap can result in poorer prediction. Two alternate data fusion strategies to improve prediction of secondary structure content will be compared and presented.

Keywords: Biospectroscopy, Chemometrics
Application Code: Bioanalytical
Methodology Code: Chemometrics
Metabolomes, the “mirror on the wall” for complex proteomes and transcriptomes show strong promise for generating new hypothesis regarding the early stages of cancer development, and for revealing biomarkers providing multivariate diagnostic indexes. Mass spectrometry-based metabolomic technologies have a demonstrated potential for early disease detection in clinical settings (e.g. newborn screening), provided the specific assays employed have the sample throughput, robustness and reproducibility necessary to handle numerous clinical samples reproducibly.

In this talk, I will discuss recent results from our team of collaborators involving the use of metabolomic workflows involving mass spectrometric measurements in serum samples coupled to support vector machine learning with the aim of identifying panels of metabolic features that can detect ovarian and prostate cancers at early stages in both human and mouse models.
Chromatographic efficiency improves with the inverse square of the particle size of the chromatographic particle. This is well documented in most theories describing band dispersion in chromatography. As the dimension of the particle decreases the pressure drop across the chromatographic column is expected to also increase. However, nanostructured materials that are not particle-based but nanofiber-based materials and are also self-organized at the macroscopic and nanoscopic level seem to be violating Darcy’s law for flow properties. This talk will illustrate substantial improvements in chromatographic efficiencies using organized nanostructures without the expect gain in enhanced pressure drop. We will also illustrate unique range of chromatographic selectivity through the use of highly organized carbon nanostructures.

Keywords: Capillary LC, Education, HPLC Columns
Application Code: Nanotechnology
Methodology Code: Liquid Chromatography
There has been an increase interest in exploring nanomaterials as chromatographic media to effect separations. We are investigating the use of graphene oxide (GO) and nanodiamond materials to modify silica supports for implementation as stationary phases in liquid chromatography. Nanodiamond particles have been hydrogenated and coupled to silica particles as well as monoliths. Our initial efforts have concentrated on the production and characterization of the nanodiamond and GO nanoparticles suitable for attachment to silica. The GO nanoparticles in particular, were investigated via chromatography and capillary electrophoresis prior to attachment to silica supports. In this presentation we will discuss the production and various characteristics of the nanomaterials, as well as their attachment to silica support. In addition, characterization of the silica modified material (e.g., TEM, FTIR, and XPS), and their initial chromatographic behavior will be presented.
DNA analysis has widespread applicability in biology, medicine, ecology, agriculture, biodefense, biotechnology and forensics. DNA separation by length is readily achieved using sieving gels in electrophoresis. Separation by sequence is less simple, generally requiring adequate differences in native or induced conformation or differences in thermal or chemical stability of the strands that are hybridized prior to measurement. We previously demonstrated separation of four single-stranded DNA (ssDNA) 76-mers that differ by only a few A-G substitutions based solely on sequence using guanosine-5'-monophosphate (GMP) in the running buffer [1,2]. Further study has revealed that it is the phosphate in the GMP that is responsible for the separation [3]. Studies of different phosphate, polyphosphate compounds as well as other salts indicate that the sequence-based separation is a much more general phenomenon that is not limited to phosphate. We are exploiting this discovery in a new, two-dimensional microfluidic platform that will use conventional sieving gels to separate ssDNA based on length in the first dimension and simple salts to separate DNA by sequence in the second dimension. Applications of particular interest that could derive important benefits from this simple, rapid separation platform include point-of-care genetic analysis for patient care and metagenomic analysis of complex microbial communities and biofilms.


Keywords: Genomics, Lab-on-a-Chip/Microfluidics, Nanotechnology, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Separation Sciences
A pre-requisite for any material used for the analysis of biological molecules is that it be biocompatible. In capillary electrophoresis proteins can adsorb onto the capillary, resulting in peak tailing, poor migration time precision and poor peak recovery. Thus, a biocompatible coating is required for bioanalysis with CE.

Often, permanent covalent coatings are used but these are time consuming and labor intensive to prepare, expensive to purchase, have limited pH stability and are difficult to regenerate. Conversely, the capillary surface may be dynamically controlled by adding a modifier to the background electrolyte. However, such additives must be present in the electrolyte to coat the wall. Their presence may alter the separation conditions and adversely affect detection.

This presentation will discuss a semi-permanent biocompatible coating that uses a surfactant bilayer as a scaffolding upon which to build a hydrophilic coating. Two-tailed surfactants such as dioctadecyldimethyl ammonium bromide (DODAB), possess a cylindrical packing factor which enables aggregation to form surface bilayers on silica. The bilayer can be stable for weeks. The surface characteristics of this bilayer scaffold are then modified by introduction of a diblock polymer. The hydrophobic block intercalates into the hydrophobic layer of the surfactant bilayer, thereby anchoring the polymer to the capillary surface. The hydrophilic block (polyoxyethylene) extends out into the solution to form the biocompatible surface.

The construct and performance of these nano-scaffolded coatings for capillary zone electrophoresis and capillary isoelectric focusing will be discussed. Such constructs form a stable coating with suppressed electro-osmotic flow over a wide range of pH (2-11). The effect of variables such as polyoxyethylene chain length, hydrophobic chain length, electrolyte pH and buffer ions on the coating stability and electroosmotic flow will be discussed.

Keywords: Capillary Electrophoresis, Nanotechnology, Protein
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Research to advance the use of smart phospholipid nanomaterials supports flexible and reprogrammable sample processing as well as tunable microscale bioseparations. This provides an innovative alternative to multifunctional separations. Phospholipids self-assemble to form 5-nanometer thick nanodisks and nanoribbons. While the thickness of the nanomaterials remains constant, the materials entangle, align, or change dimension. This is significant to biomolecule separations because these nanomaterials are a tunable scaffold to regulate the selectivity. Nanogels are integrated into microscale separations to demonstrate gradient gel separations, fluid steering, and integrated enzyme processing. Separations can be adjusted to accommodate different samples. Glycans separated based on hydrodynamic size generate theoretical plate counts of 600,000. DNA separated based on chemical sieving, yield theoretical plate counts ranging from 0.9 to 2 million. The method is used to probe biomolecules for structural information through microscale sequencing. Finally, complex samples, are simplified using integrated serial processing to generate electropherogram that can be easily interpret.

Keywords: Bioanalytical, Biological Samples, Capillary Electrophoresis, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Separation Sciences
Synchrotron radiation has enabled major discoveries in structural biology over the past 4 decades. Technology developments and innovations have been essential in making this possible, most notably in robotics, software control and detectors. Recently, a new x-ray light source, the LCLS x-ray free electron laser at SLAC, has opened another new avenue of investigation where the unique properties of the LCLS x-rays (fsec pulses, extreme brightness and coherence) have enabled experiments not feasible with ‘conventional’ synchrotron sources. This talk will provide an overview of recent developments and pioneering research for both conventional and free electron laser light sources.

The operation of SSRL and LCLS at SLAC is supported by the U.S. Department of Energy, Office of Basic Energy Sciences and support in structural biology R&D is provided by DOE Office of Biological Research and the National Institutes of Health Institute of General Medical Sciences (NIGMS).

Keywords: Biotechnology, Protein, X-ray Diffraction
Application Code: Biomedical
Methodology Code: X-ray Techniques
“Footprinting” refers to assays that examine ligand binding and conformational changes by monitoring the solvent accessibility of the backbone (nucleic acids) or side chains (proteins) of macromolecules through their sensitivity to chemical or enzymatic cleavage or modification reactions. The folding, ligand binding, assembly, and conformational dynamics of macromolecules, the details of which are intimately revealed by footprinting assays, are critical to understanding their functions in a diverse range of activities such as splicing, signaling, catalysis, ion flux, replication, transcription and translation. Synchrotron X-ray footprinting employs the intense and ionizing white x-ray beams produced by synchrotron radiation to generate hydroxyl radicals in solution on the microsecond to millisecond timescales appropriate for probing macromolecular structure and dynamics while minimizing sample perturbation. Enhancing the flux density of white-light radiation in the range of 5-20 keV on the sample is the key figure of merit for enhancing the quality and accessing shorter timescales for synchrotron X-ray footprinting experiments. The X28-C beamline at the National Synchrotron Light Source (NSLS) has been the leading facility for conducting X-ray footprinting since its construction in 2000. Improvements in flux density at X28-C over its lifetime have permitted structure assessment of protein complexes and membrane protein systems of great biological interest. With the pending shutdown of the NSLS and the construction of NSLS-II, we have secured funding from NSF and NIH to build XFP, a new generation footprinting beamline. This talk will highlight the advanced capabilities of XFP and the novel structure and dynamics studies that it will enable when it comes on line in late-2015.

Supported by NIH-P30-EB09998 and NSF-DBI-1228549
Macromolecular crystallography (MX) is the most powerful method capable of providing information about atomic structure and interactions. New synchrotron sources and dedicated MX beamlines have expanded our competence in structural biology. Novel approaches allow data collection from demanding crystals using mini-beams and reduce radiation damage. Genome sequencing has enlarged protein sequence space and allow for comprehensive studies of cellular systems. Structural Genomics efforts contributed a complementary array of rapid and cost effective methods. When combined with MX synchrotron facilities, advanced software and computing resources, these methods resulted in acceleration of protein structure determination, improved quality and have a wide range of applications.

The introduction of antibiotics in medicine gave false hope for the control of infectious diseases. Today antibiotic resistance is discovered against key antibiotics worldwide. The NDM-1 enzyme makes pathogenic bacteria resistant to all known [beta]-lactam antibiotics. NDM-1 represents an example of extreme promiscuity - it can hydrolyze a wide range of [beta]-lactams; it can utilize different metal cofactors and seems to exploit alternative mechanisms. The structures of NDM-1 in complex with ligands revealed an enlarged and flexible active site capable of accepting many substrates and aid mechanistic and inhibitor studies.

The development of new antibiotics that are effective against drug-resistant strains and the discovery of new drug targets are equally important. The IMPDH controls the GMP pool and is often found essential. Recent progress on specific IMPDH inhibitors has prompted a new interest in bacterial enzymes as drug targets. Structural studies of bacterial IMPDHs in complex with inhibitors combined with binding studies provide insight to how species-specific inhibitors can be developed.

Work was supported by NIH (GM094585 and Contract #HHSN272200700058C) and the DOE/Berkeley (DE-AC02-06CH11357).
The ability to modify metabolic processes induced by either genetic alterations or environmental stimuli is critical for maintaining an efficient functional cell. Much of our qualitative and quantitative understandings of cellular pathways at a molecular level has come from traditional biochemistry experiments that are averaged over large populations as well as performed in vitro with purified biomolecules. Although these approaches have clarified many detailed mechanisms, they have difficulty elucidating the phenotypic differences that exist even within a genetically homogeneous population. A pervasive key challenge ranging across microbial ecology, bioenergy, and biomedicine is to identify role-defining cells within a large population and to monitor their real-time biochemistry in situ.

In this presentation, we highlight advances that now allow infrared spectromicroscopy to address these tasks and present application examples. Central to this approach is the use of the synchrotron light source, which enables high-throughput noninvasive spectroscopic microanalysis. This capability can precisely target subpopulations with a diffraction-limited spatial resolution while accurately tracking their unlabeled chemical reactions with molecular specificity—our currently acquirable timescales associated with a number of important biological processes.

Keywords: Biospectroscopy, Biotechnology, FTIR, Microspectroscopy
Application Code: Bioanalytical
Methodology Code: Biospectroscopy
Structural biology has undergone tremendous developments during the last decades, allowing the determination of high-resolution structures at the atomic level of some of the largest protein complexes known to date, both soluble and integrated into biological membranes. These developments have become possible due to the construction of most advanced synchrotron radiation facilities around the world, and more recently, even powerful free electron lasers. These infrastructure are serving as a platform for rapid advance in terms of experiment throughput, automation and versatility of experiment design. In this presentation, ongoing developments will be reviewed and future perspectives will be outlined.

Keywords: Biological Samples, Protein, X-ray Diffraction
Application Code: Biomedical
Methodology Code: X-ray Techniques
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. Recently, a robust proof that tip-enhanced Raman spectroscopy (TERS) is capable of single molecule sensitivity was carried out using the Rhodamine 6G (R6G) isotopologue method previously demonstrated for single molecule SERS. The overall enhancement factor was found to be $10^{13}$. This was further partitioned into an electromagnetic contribution of $10^6$ and a resonance Raman contribution of $10^7$.

Substantial progress in ultrahigh vacuum (UHV) TERS has been achieved by adding low temperature capability (LT). LT (19K)-UHV-TERS experiments have been carried out on the R6G/Ag tip/Ag(111) system. Dramatic line width narrowing was observed in LT-UHV-TERS compared to room temperature (RT)-UHV-TERS revealing new Raman lines and spectral shifts due to the interaction of R6G and Ag(111). Spectral shifts were observed mainly for modes localized on the xanthene moiety, while the modes associated with the pendant phenyl ring were unaltered. The high sensitivity and narrow line widths of LT-UHV-TERS provides new window into adsorbate surface interactions.

LT-TERS also opens new horizons for the application of this technique to biological problems. Specifically, we anticipate that the structures of transmembrane proteins may be directly obtained using LT-TERS. Moreover, coupling of the freeze plunger technique, which is commonly used in cryo-SEM, with TERS should allow for imaging whole cells and microorganisms at the nanoscale.

Keywords: Biospectroscopy, Materials Science, Spectroelectrochemistry, Vibrational Spectroscopy
Application Code: Biomedical
Methodology Code: Biospectroscopy
Tip-enhanced Raman scattering (TERS) is becoming a tool for the investigation of biomolecules with a lateral resolution beyond 10 nm. In the best resolution cases only parts of larger molecules actually contribute to a spectrum, rendering the spectral features different from bulk information. In this paper we will discuss the influence of the high resolution (both lateral and vertical) towards the spectra and how this has to be considered for a reliable assignment of spectral data. As examples TERS investigation of complex biomolecules will be compared to model systems where the environment can be easily controlled.
Collagens are the most abundant proteins in the human body and the main constituent of the extracellular matrix surrounding each cell in a tissue. In vitro and in vivo, collagen molecules assemble through a hierarchical process into fibrils with a diameter up to 500 nm. These fibrils act as a chemical and mechanical scaffold that is essential for the development and maintenance of living tissues. As tissues develop and ages the collagen fibrils are modified through chemical cross-linking, through the addition of different collagen types and through binding of proteins and proteoglycans. In this context, tip-enhanced Raman spectroscopy (TERS) has both the chemical sensitivity, surface sensitivity and spatial resolution to map chemical modifications at the surface of single collagen fibrils. As a proof of principle we have used both SERS and TERS to characterize the surface of collagen I fibrils assembled in vitro from acid solubilized collagen. We demonstrated that most of the signal comes from interactions between the Raman probe and phenylalanine residues at the surface of the fibrils. This study highlighted the challenges associated with TERS on large and "soft" supramolecular assemblies such as collagen I fibrils.
Membrane Receptors Probed with Tip Enhanced Raman Scattering

Tip enhanced Raman scattering (TERS) is a chemically specific method that provides increased sensitivity and spatial resolution for optical microscopy. Electromagnetic enhancement of the Raman signal is generated from molecules within the evanescent field of a plasmonic nanostructure on the end of an atomic force microscope, providing Raman images with spatial resolution proportional to the tip curvature. The field is further enhanced when the TERS tip is near a metal surface, or a second nanostructure. In this presentation we will discuss how that small molecules with specific binding affinity for membrane receptors can direct the adsorption of nanoparticles onto intact cellular membranes to provide chemical information about the receptor protein. The functionalized nanoparticle serves a double purpose: 1) providing a beacon on the membrane landscape to identify the location of the receptor and 2) increasing the TERS response of the amino acids near the nanoparticle. The signals we have observed indicate the protein does not have to reside in the gap junction for high sensitivity detection. Further the reproducibility of the signal provides insight into the ligand-receptor specificity. This methodology has potential to investigate the chemical properties associated with cell signaling, adhesion, and drug delivery.

Keywords: Bioanalytical, Biospectroscopy, Raman, Surface Enhanced Raman
Application Code: Bioanalytical
Methodology Code: Biospectroscopy
Biological TERS: Instrumentation Development and Applications

TERS is Uniquely Suitable for Structural Characterization of the Surface of Amyloid Fibrils

Amyloid fibrils associated with many neurodegenerative diseases are the most intriguing targets of modern structural biology. Significant knowledge has been accumulated about the morphology and fibril-core structure recently. However, no conventional methods could probe the fibril surface despite its significant role in the biological activity. Tip-enhanced Raman spectroscopy (TERS) offers a unique opportunity to characterize the surface structure of an individual fibril due to a high depth and lateral spatial resolution of the method in the nanometer range. Here, TERS is utilized for characterizing the secondary structure and amino acid residue composition of the surface of insulin fibrils. It was found that the surface is strongly heterogeneous and consists of clusters with various protein conformations. The propensity of various amino acids on the fibril surface and specific surface secondary structure elements were evaluated. The comparison of surface properties for two different insulin fibril polymorphs prepared at different pH and its kinetic preciouses (protofilaments) will be discussed. Overall, this study provides valuable new information about the structure and composition of the insulin fibril surface and demonstrates the power of TERS for fibril characterization.

Keywords: Biospectroscopy, Microscopy, Raman, Surface Enhanced Raman
Application Code: Nanotechnology
Methodology Code: Microscopy
In this talk I will recollect the problems and tribulations I went through when I started Applied BioPhysics, Inc. together with my friend Dr. C.R. Keese. Applied BioPhysics manufactures scientific instruments used by scientists studying cells in tissue culture. It is becoming more and more important for scientists or engineer to translate a scientific invention or discovery into a successful business, and that was what we tried as well. A unique method and appropriate instrumentation to quantify the behavior of cells in tissue culture has been developed in our laboratory over the last two decade. The technique is referred to as Electric Cell-substrate Impedance Sensing (ECIS). The basic principle of the method is to culture mammalian cells on small gold electrodes. When cells attach and spread on these electrodes, the measured electrical impedance changes because the cells constrain the current flow. By monitoring the impedance of the cell covered electrodes, the morphology and motion of the cells can be inferred in real time. Since these behaviors, such as spreading and locomotion, involve the coordination of many biochemical reactions, they are extremely sensitive to most external parameters including temperature, pH, and a myriad of chemical compounds. This broad response to changes in the environment allows this method to serve as a general biosensor. The measurements are easily automated, and the general conditions of the cells can be monitored by a personal computer controlling the necessary instrumentation.
The U.S. Army Center for Environmental Health Research is developing a dual system of field portable toxicity sensors for rapid response (within 90 minutes) to a broad spectrum of industrial and agricultural chemicals that have the potential to contaminate Army field drinking water supplies. The sensors include an electric cell-substrate impedance sensing (ECIS) system, which uses fluidic biochips seeded with rainbow trout gill (RTgill-W1) cells, and a pesticide detection kit designed specifically to detect organophosphate and carbamate chemicals. The combination of the two technologies can detect a wide range of representative chemicals at concentrations that can cause adverse health effects. The pesticide detection sensor consumables have a shelf-life of at least 9 months at room temperature, while the ECIS sensor biochips containing RTgill-W1 cells can be stored at 6 °C with no media replenishment for over a year and still respond to benchmark toxicants. This presentation will describe the development and utility of the ECIS sensor as a field portable water toxicity sensor, as well as data on its sensitivity to chemical detection.

Disclaimer: The views, opinions, and/or findings contained in the report are those of the authors and should not be construed as official Department of the Army position, policy, or decision, unless so designated by other official documentation.

Keywords: Biosensors, Detection, Environmental/Water, Sensors
Application Code: Environmental
Methodology Code: Sensors
The maintenance of cell volume is essential for life. Thus, monitoring cell volume is a powerful tool for the global influence of environmental factors. While Coulter counters have long been used to evaluate cell volume of suspended cells, adherent cells, the most common variety, regulate volume differently than suspended cells. We have developed a variety of impedance-based microfluidic chips to accurately measure cell volume in adherent or suspended cell populations and single cells in real time at high resolution, sensitivity and at low cost. Time resolution is important since the rate of change of cell volume is related to membrane permeability and to the cell metabolism that regulates volume. Given that the cell volume changes are the integral of the water flux, the volume sensor has been used to measure water flux across cell membrane and screen chemicals that affect water permeability. We have demonstrated that Hg2+ and other heavy metals affect water transport via aquaporins (AQPs). Cells respond to extracellular osmotic pressure by transient changes in volume, and then via a variety of regulation mechanisms return cells towards the resting volume. We have measured the effect of a variety drugs on cell volume regulation and the rates at which cell respond to osmotic challenges. We have been able to show that some cells use mechanosensitive ion channels to regulate volume and some cells don’t. The sensors also allow for rapid variations in shear stress as well as rapid solution changes and simultaneous optical observation for fluorescent probes. The system is easily parallelized to arbitrary complexity. We have shown that shear stress alone reduces cell volume, and this involves pharmacologically undefined pathways. Recently, we have expanded the system to measure ion diffusion and electrical resistance within cell populations. Impedance provides a probe free, fast, nondestructive, inexpensive and accurate tool to measure a variety of cell properties.

Keywords: Biosensors, High Throughput Chemical Analysis, Lab-on-a-Chip/Microfluidics, Sensors
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Abstract Text
Aptamers are DNA and RNA oligonucleotides that can bind to a variety of non-nucleic acid targets with high affinity and specificity. Pathogen detection is a promising area in aptamer research. One of its major advantages is the ability of the aptamers to target and specifically differentiate microbial and viral strains without previous knowledge of the membrane-associated antigenic determinants or molecular biomarkers present in that particular microorganism. This presentation summarizes our recent developments of electrochemical aptamer-based sensors for microbial and viral pathogen detection, viability assessment of microorganisms, bacterial typing, identification of epitope-specific aptamers, affinity measurement between aptamers and their respective targets, and estimation of the degree of aptamer protection of oncolytic viruses for therapeutic purposes.

Keywords: Bioanalytical, Biosensors, Electrochemistry, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Electrochemistry
IAEAC: Label-Free Biosensing: Impedance-Based Biosensors for Environmental Applications

Hyphenated Impedimetric Sensors: A New Route to a Non-Imaging, Label-Free High Content Screening?

Label-free experimental approaches suitable to continuously monitor animal cells in vitro provide an entirely new perspective on cell-based assays as performed, for instance, in drug development or cytotoxicity profiling. The independence of labels together with non-invasive readout principles provides the technical requirements for (i) continuous and longterm cell observation, (ii) acquisition of unbiased information about the cells under study and (iii) running several sequential assays on one and the same cell population.

Among the available label-free approaches impedance analysis of cells grown on conducting surfaces is the farthest developed and referred to as 'electric cell-substrate impedance sensing (ECIS)'. ECIS uses non-invasive AC currents to measure the impedance of cell-covered electrodes which is dominated by the ionic current pathways around and through the cells. Thus, the time course of impedance reports on changes in cell shape with nm accuracy providing a time resolution between milliseconds and weeks. Using changes in cell shape as a very general analytical indicator makes ECIS broadly applicable. Fields of application comprise cytotoxicity studies, apoptosis monitoring, signal transduction or cell migration, to mention a few. The biggest disadvantage of impedimetric monitoring is its lack of molecular specificity or the ease of unspecific perturbations of the signal. This presentation will highlight how impedance analysis of cell-covered electrodes can be coupled to other label-free analytical techniques like the quartz crystal microbalance, surface plasmon resonance spectroscopy or potentiometric recordings. The combination of techniques (hyphenated ECIS) increases the information depth and provides a more comprehensive, time-resolved view on the cells while they are exposed to a stimulus of interest. As such hyphenated impedimetric monitoring has the potential to be the basis for a non-imaging high content screening approach.

Keywords: Bioanalytical, Biosensors, Electrode Surfaces, Environmental Analysis

Application Code: Bioanalytical
Methodology Code: Sensors
The Curiosity rover that landed in Gale crater in 2012 carried the first laser-induced breakdown spectroscopy (LIBS) experiment to Mars. LIBS is part of the ChemCam package which also includes the Remote Micro-Imager (RMI), providing the highest resolution remote imaging on Mars. ChemCam uses a Nd:KGW laser and a 110 mm telescope to direct 14 mJ pulses on target, obtaining remote chemical analyses and depth profiles to 7 m distance with a 400 micron beam. In normal operation RMI images are taken before and after LIBS. Typically 30 laser pulses are used per observation point, and points are arranged in a line of 5-10 points or a grid of 9 or 16 points. A 6144-channel spectrum spanning 240-850 nm is returned from each pulse. In addition to quantification of the major elements, minor and trace elements H, Li, Mn, Cr, Rb, Sr, Ba are easily observed. The LIBS acts as a microprobe, observing individual mineral grains in coarse-grained rocks. ChemCam showed for the first time that Mars dust is hydrated, accounting for a large fraction of the surface H observed globally from orbit by neutron and gamma-ray spectroscopy. ChemCam also discovered for the first time local components in soils in the form of fine < 1 mm pebbles. Akali feldspar-rich rocks were observed in surface gravels and conglomerates near Bradbury Landing site. Ca-sulfate veins and Mg- and Li-enriched ridges were identified at Yellowknife Bay (sol ~120). These record secondary alteration of the mudstones discovered at the lower, and apparently once freshwater-filled, part of Gale. On sol 325 (June 2013) Curiosity began a 9 km trek to Mt. Sharp, a 5 km tall sedimentary mound in the center of Gale where hematite- and clay-rich signatures are seen from orbit. Nearly daily LIBS analyses provide a record of changes in rock and soil compositions along the traverse. Overall, ChemCam provides a large and extremely rich dataset supporting the Curiosity rover’s identification of habitable environments on the Red Planet.
Laser-Induced Breakdown Spectroscopy (LIBS) as an Emerging Tool: Figures, Facts and Future

Laser-Induced Breakdown Spectroscopy (LIBS) is a method of optical emission spectroscopy that uses laser-generated plasma as the source of vaporization, atomization and excitation. A bibliographic study around the LIBS literature shows clearly the number of application areas related to LIBS and laser based techniques is still growing. There is no doubt that LIBS has become a fascinating technology with great promise. The benefits include no sample preparation, no consumables, every sample, real-time analysis, standoff measurements, and more. LIBS provides ppm sensitivity for elemental analysis and even offers molecular characterization based on database libraries and chemometrics.

In the last decade, there has been a significant technological developments in the components (lasers, spectrometers, detectors) used in LIBS instruments as well as emerging needs to perform real time measurements under conditions to which conventional techniques cannot be applied. This opens the door for many applications and possibilities of developing field-deployable instruments.

Recently, fiber lasers have become one of the hottest topics in photonics. In this presentation, we will report their use for LIBS analysis and will discuss their advantages and inconvenient. Also, we will give an overview about LIBS applications for real time analysis, we will discuss the LIBS instrumentation in terms of robustness, analytical performance and portability in comparison to conventional techniques. In addition, we will present some approaches to improve the LIBS sensitivity developed in our laboratory and elsewhere, such as laser-induced fluorescence coupled to LIBS, resonance enhanced LIBS, resonant ablation, etc.

Keywords: Atomic Spectroscopy, Chemical, Laser, Sensors
Application Code: Process Analytical Chemistry
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Application of Laser Induced Breakdown Spectroscopy (LIBS) to measure gas composition will be presented. Two important applications will be described in detail. First is an analytical LIBS sensor to provide a state-of-art, near real-time measurement of several of the most critical impurities that are present in hydrogen fuel. A LIBS system is designed to detect trace helium (He), argon (Ar), nitrogen (N) and oxygen (O) in hydrogen gas (H2 concentration > 99%). The effects of laser energy, the delay time between the laser pulse and data acquisition, and the gas pressure on the LIBS signal intensity of H (656.3 nm), Ar (763.5 nm), N (746.8 nm), O (777.5 nm) and He (587.56 nm) were investigated to determine the optimum conditions for detection. The broadening of the hydrogen alpha line (656.28 nm) was found to increase as the concentration of helium impurities in the hydrogen gas mixture increased. The quenching of the He emission due to Penning ionization was observed at He concentrations above 7.25%. However, the LIBS signal from the helium 587.56 nm line shows good linear correlation with helium concentration for He concentrations below 1%. The limit of detection (LOD) for He, Ar, N, and O in H2 were found to be 83, 36, 37, and 11 ppm, respectively. A LABVIEW software program was developed to control data acquisition, perform data analysis and display real-time data. The second LIBS application to gas sample analysis is to measure carbon dioxide (CO2) in the atmosphere. The C(I) emission line at 247.85 nm was selected for calibration and validation in LIBS measurement of CO2. The effects of laser energy, the delay time between the laser pulse and data acquisition, and the gas pressure on the LIBS signal intensity were investigated to determine the optimum conditions for CO2 detection. The limit of detection (LOD) for CO2 in air was estimated to be 47 ppm.

Keywords: Atomic Emission Spectroscopy, Gas
Application Code: Biomedical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The U.S. Department of Energy’s carbon capture and sequestration (CCS) program goals to reduce the emission of CO2 from anthropogenic sources will entail a great amount of cost and efforts and deep saline formations have great potential for geologic CO2 sequestration. To ensure the success of the program it is important that the carbon dioxide that is injected underground remains there (99% permanence over 1000 years). A number of CO2 monitoring techniques have been employed since the inception of the CCS program. The methods range from the injection of tracers, micro-seismic monitoring techniques, satellite imaging, aerial monitoring with gas sensors, and various optical techniques. We propose the use of laser induced breakdown spectroscopy (LIBS) analytical technique for evaluating potential leaks (i.e., CO2 and brine leakage) from the storage sites. The chemical composition of brines varies greatly by location and typically sodium is the most abundant element making 70% to 90% of total cation mass. We are using LIBS to examine the effects of sodium chloride (NaCl) molar concentration on the LIBS signals of Li, Ca, Mg, K, and Sr. Solutions of the chloride salts were prepared by varying the molar concentration of NaCl in each solution ranging from 0 to 3 M. A Q-switched Nd:YAG laser with a 9 ns pulse length operating at 1064 nm was used to produce plasma in bulk solutions. The plasma emission was spectrally analyzed and the signals from the analytes were investigated to understand the enhancement that high concentrations of sodium had on the overall measurement. The study has implications in developing LIBS technique for examining changes in fluid composition resulting from interaction of CO2 with characteristic brine reservoirs.

Keywords: Environmental, Environmental Analysis, Environmental/Water, Laser
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Abstract Text
In this talk we will demonstrate the ability of laser-induced breakdown spectroscopy (LIBS) for in-situ analysis of a variety of gallstones, and kidney stones. For recording the LIBS spectra, we have directly used these stones after surgery of the patients belonging to the different regions of India. After surgical removal, the stone samples were washed with deionized water to remove the blood and other adherent contaminants and brought to the laboratory in sealed pots. In some cases the stones were cut into two pieces using a clean, sharp blade and further cleaned to remove surface contaminants. LIBS spectra of these stones were recorded by focusing directly the laser beam on the surface of these stones. The identification of elements is based on the presence of persistent lines of the elements which can be observed even at the lowest possible laser irradiance. All the elements present in the stones were identified by spectral analysis using the National Institute of Standards and Technology (NIST) database. The presences of mineral composition in the stones are correlated to the dietary habits and culture of the people/patient. In addition to the atomic spectral signatures of elements, molecular bands such as C2 swan bands and CN bands have also been used to differentiate the stones. We have successfully discriminated the different layers (dark and light layers) of gallstones on the basis of the presence and intensities of the spectral lines for carbon, hydrogen, nitrogen, oxygen and copper. Finally, LIBS in combination with PCA has been used for rapid identification/classification of the different types of stones samples.

Keywords: Atomic Emission Spectroscopy, Biomedical, Statistical Data Analysis, Trace Analysis
Application Code: Biomedical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Refining Chemical Analysis in the Central Nervous System

In-Vivo, Real-Time Chemical Characterization of Brain Tumour Tissues by Rapid Evaporative Ionization Mass Spectrometry

In-situ tissue identification is highly important during the surgical resection of the malignant tumours of the central nervous system. Residual tumour tissue (especially in case of gliomas) leads to rapid and aggressive recurrence, while damage to healthy tissue may result in loss of key physiological functions. Solution for the problem of in-situ tissue identification has been sought for decades, and a number of different solutions have been proposed including intraoperative medical imaging, in-vivo labelling of tumour tissue and various spectroscopic approaches including mass spectrometry (MS).

Direct MS analysis of tissue sections by various desorption ionization (DI) methods yields histologically specific data, which served as a basis for developing MS technologies for the in-vivo analysis of tissues. While existing DI methods were proven to be incompatible with the surgical environment, traditional surgical diathermy (electrosurgery) was discovered to work as an ion source by converting certain molecular components of tissue to gas-phase ions. The resulting method was termed ‘Rapid Evaporative Ionization MS’ referring to the critical nature of the rate of evaporation regarding ion formation. Since diathermy is used in neurosurgery in the form of bipolar coagulation, bipolar surgical forceps were coupled with mass spectrometry to obtain an analytical device capable of in-vivo, quasi real-time (time delay ~ 1 s) tissue characterization. The experimental setup was successfully tested ex-vivo, and multivariate statistical analysis of data revealed good histological specificity. The device has also been successfully tested in-vivo in a cohort of 81 patients with post-operative histological validation. The results revealed that most malignancies of the CNS can easily be identified intraoperatively using REIMS technology and the approach combined with neuronavigation may provide a real alternative to currently used technologies.

Keywords: Instrumentation, Lipids, Mass Spectrometry
Application Code: Biomedical
Methodology Code: Mass Spectrometry
Traumatic brain injury (TBI) is caused by a trauma to the head and the effects can be devastating. 40% of patients develop a secondary injury leading to a more severe outcome. At present it cannot be predicted who will develop secondary injury. Spreading depolarisations (SD waves) have been detected in the injured human brain and have been characterised using rapid-sampling microdialysis [1]. Notably, during the SD wave the glucose levels decrease and lactate levels increase. Repetition is seen in some patients, driving the local glucose levels down, possibly to a level where the tissue is no longer viable [2]. It has been found that the presence of SD waves, particularly if in clusters, is linked to a poor patient outcome.

To detect these dynamic changes on-line and in real-time, we are combining microdialysis with microfluidic platforms [3]. Microfluidics easily handles the small sample volumes produced when using microdialysis. Electrodes have been designed for a microfluidic analysis chamber [4] and can be fabricated into electrochemical biosensors to detect levels of glucose and lactate. The problems and possible solutions of combining sensors, microfluidic platforms and an automated calibration system, into an instrument designed for clinical use, will be discussed.

References:

Keywords: Electrochemistry, Lab-on-a-Chip/Microfluidics, Neurochemistry
Application Code: Neurochemistry
Methodology Code: Microfluidics/Lab-on-a-Chip
Micro-fabricated neural electrode arrays, placed in the nervous system to directly interface with neurons, have tremendous research and clinical significance. However, current intracortical neural electrodes arrays experience recording failure including signal drift and degradation due to biochemical, mechanical and electrical mismatch between the artificial device and brain tissue. To understand the cellular and tissue response at the neural electrode-tissue interface, multimodal analysis was done using a combination of postmortem immunohistochemistry, live-animal multi-photon imaging and neurophysiological recording. It was observed that implantation of microelectrodes cause immediate vascular damage and microglial activation followed by inflammatory gliosis and chronic neural degeneration in the brain tissue. Furthermore, several bioengineering strategies have been developed to modulate the brain tissue response towards a seamless and stable neural electrode-tissue interface. The first strategy is to immobilize biomolecules onto the implant surface to promote the growth and attachment of neurons while suppressing the glial cell reaction. Neural electrodes coated with neural adhesion molecule L1 showed enhanced neuronal ingrowth and minimized microglia reaction on and around the implant. Secondly, an on-demand release coating that can actively deliver anti-inflammatory or neuroprotective drugs is being developed. This coating takes advantage of the electrically conductive and electroactive conducting polymer and carbon nanotubes, which allow the drug molecules to be loaded and electrically released while enhancing the recording and stimulation capabilities of the electrodes. Thirdly, electrode arrays that are ultra-small and/or ultra-compliant have been developed to minimize the brain tissue response.
Abstract Text

The ability to monitor neurotransmitter release in freely behaving animals is key to understanding neuronal processes underlying complex behaviors. Such behaviors are controlled by neuronal networks employing multiple neurotransmitters and neuromodulators. Efforts to understand how neurons within these networks interact to control behavior will be greatly facilitated by a means with which to measure multiple neuroactive molecules simultaneously and in near-real time. Existing methods either offer rapid measurements of a single analyte (e.g., fast-scan cyclic voltammetry) or provide multiple analyte measurement with insufficient temporal resolution (microdialysis). Our goal is to develop an implantable microprobe capable of simultaneous rapid monitoring of 3 ubiquitous neurotransmitters/neuromodulators: dopamine (DA), glutamate (Glut), and acetylcholine (ACh), while optogenetically modulating their release with onboard optics. Progress towards this goal will be presented. We have also begun to employ basic versions of these sensors to measurement of glutamate in the brain during performance of goal-directed instrumental actions. We have observed rapid, transient Glut concentration changes in the amygdala during the decision-making period prior to reward-seeking actions. Moreover, these transient Glut signals were found to be of neuronal origin and to significantly correlate with reward-seeking activity, providing valuable information concerning the role of Glut in these behavioral processes.

Keywords: Biosensors, Electrochemistry, Electrodes, Sensors
Application Code: Neurochemistry
Methodology Code: Sensors
The Mayo Neural Engineering Laboratories has been studying the mechanism of action of DBS using fast scan cyclic voltammetry. Previously, we developed a neurochemical monitoring device called WINCS (Wireless Instantaneous Neurochemical Concentration Sensing system), and confirmed its functionality for fast scan cyclic voltammetry (FSCV) monitoring during animal and human DBS neurosurgery. To expand the utility of the original device, we developed a novel wirelessly controlled stimulation device called Mayo Investigational Neuromodulation Control System (MINCS). In addition, a 4 channel electrometer combined with stimulation device called WINCS Harmoni, a third generation device which provides wirelessly controlled stimulation interleaved with 4 channel FSCV, amperometry or electrophysiology. In animal (rat) testing, WINCS Harmoni evoked dopamine release detectable by FSCV electrode at the striatum, as measured by simultaneous and interleaved FSCV. Importantly, the controlled release of dopamine was detected without stimulation artifact during the application of variable and wirelessly controlled high-frequency stimulation. Allowing such ongoing monitoring of neurochemical changes during the stimulation period may pave the way towards future closed loop DBS systems.
The research and advances in analytical chemistry are essential to ensure quality of life. The theme of the 17th National Meeting of Analytical Chemistry, held in Brazil in 2013, was Analytical Chemistry and Quality of Life. In this context you will see an overview of the main contributions of Brazilian researchers in the development of analytical methods that are directly or indirectly related to improving the quality of life of people. With the data presented is intended to also show how the Analytical Chemistry in Brazil has advanced and what are the prospects for the coming years.
São Paulo is the richest state of Brazil, producing more than 33% of the GDP of the country. Its territory area occupies less than 3%, but its population corresponds to more than 42 millions, or 22% of inhabitants of the country. The State finances three large State Universities - USP, Unicamp and Unesp, which receive 9.57 % of all taxes incomes – so as Fapesp, the São Paulo State Research Foundation that receives 1% of all taxes collected. This “recipe” promoted an intense growing of the research capacity in the State. The three State Universities together with the Federal universities and Research Institutes are responsible for more than 1% of all the science published worldwide. To attain this condition, it was necessary create a solid infrastructure that favors the development of research of high quality. To joint this public system, the students are free of fees and can apply for fellowships from Fapesp, from the Brazilian National Research Council (CNPq) or for the Coordination for the Improvement of higher level staff (CAPES). For “hard sciences”, in special chemistry, there are many facilities to produce excellent research in all the universities. In our presentation we will show these aspects and discuss the opportunities of research in São Paulo.

Keywords: Analysis
Application Code: General Interest
Methodology Code: Education/Teaching
Analytical Chemistry Opportunities in Areas of Interest

Petrobras is an open capital company, whose main shareholder is the Brazilian government. The company is leader in the oil sector in Brazil. As an integrated company it operates in the sectors of exploration and production, refining, marketing and transportation of oil and natural gas, and in the distribution of petrochemical and derivatives. The research on new sources of energy, such as biofuels and other renewable sources are thus part of the company’s business portfolio. To overcome the many challenges in the energy research projects the company has developed a model of partnerships with universities and research institutes. This is done through collaborative networks, with the goal of capacitating human resources and improving the infrastructure in specifics areas of interest. This effort involves 101 Brazilian universities and 49 thematic networks. On top of that, Petrobras is supporting, until 2017, the Brazilian Program science beyond borders with 5.000 graduation and post-graduation scholarships. These scholarships aim for international exchange in themes related to oil and gas, energy and biofuels industry. Analytical Chemistry research presents opportunities in all areas of interest. People with different knowledge and background can share information and experience to ensure that our scientific community is in the best position to face the challenges in their projects. As a final result the company and its partner institutions celebrate the success towards innovation and excellency in the themes mentioned above.

Keywords: Fuels\Energy\Petrochemical
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Chemical Methods
From the viewpoint of industrial sector, we believe that never before has the interaction between industries and universities through cooperation programs, partnerships and academic exchanges been in such evidence. Topics such as energy, renewable raw materials, green chemistry, life sciences, food safety and innovation permeate all economic sectors. It means important and urgent demand for qualified human resources with creativity and comprehensive vision of processes and products. In this scenario what are the opportunities for Analytical Chemists and what is the paper of the professional in the market?
Science in Brazil has had a significant development in recent years. Thus, Brazilian institutions need to rapidly engage in the process of internationalization which has been experienced by other nations in the same sector. To overcome hindrances towards the forging of a more comprehensive and international view on science, Brazil has created a Scientific Mobility Program – CSF – a government sponsored initiative with the goal of promoting, consolidating and expanding science, technology and innovation in the country. Through partnerships with top international educational and research institutions throughout the world, the CSF has increased the presence of students and scientists from universities and representatives from our national industry in such countries and institutions, as an effort to expand the required knowledge to help booting the sector in the national scenario. Additionally, the program seeks encouraging young talents and highly qualified researchers from abroad, to work with local investigators in joint projects, stimulating the process of internationalization of universities and research centers in Brazil. By promoting the establishment of international partnerships, the project encompasses a number of varied scientific strategic fields including, mineral, aerospace, pharmaceutical science, renewable energy, and pure and natural sciences. It is expected that until 2015, Brazil Scientific Mobility Program will grant over a hundred thousand fellowships in to Brazilian students and researchers, at the most renowned S&T institutions worldwide and to qualified researchers from those institutions who wish to work together with Brazilian scientists, as part of the concept of knowledge exchange. Through these actions, the program will have a definite impact at starting a new Era for the R&D system in Brazil, exposing students and researchers of strategic fields to a whole new range of possibilities and an environment of high quality competitiveness and entrepreneurship. This presentation will show an overview of the CSF program and also how foreigner researchers can find opportunities for collaboration with Brazilian scientists.

Keywords: Education
Application Code: General Interest
Methodology Code: Education/Teaching
Many mass spectrometry-based biomarker discovery efforts undertaken to date have utilized shotgun bottom-up quantitative proteomic analysis. In this report, we leverage high-throughput top-down proteomics for a novel take on biomarker discovery. We have developed a label-free approach using a hierarchical linear model to quantify the relative abundance of intact proteins and have employed our strategy on a set of model organisms to enable the quantitation of clinical isolates of circulating immune cells from healthy transplant patients and those patients undergoing acute cellular rejection. Our workflow represents a novel approach to biomarker studies and is the first to offer complete protein characterization and relative quantitation within the same experiment.

Keywords: Bioinformatics, Data Analysis, Proteomics, Quantitative
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Liquid Chromatography/Mass Spectrometry
Advanced research designed to expand the use of engineered proteins as biopharmaceutical agents, to understand drug engagement with targets, and to identify biomarkers has accelerated the development and integration of sophisticated methods to quantify proteins in biological matrices. Alternative hybrid methods to Ligand Binding Assays (LBA) are required to support complex discovery activities. Increasing requests for protein analysis in early discovery, coupled with rapid method development is needed in a variety of experiments and biological samples. Although LBA is considered the method through the years, it has limitations (obtaining reagents with binding characteristics including affinity and selectivity) that preclude it as the only method used. Liquid chromatography mass spectrometry is an invaluable platform in pharmaceutical research in the simultaneous determination of multiple analytes (drugs, metabolites, endogenous biomarkers). By capitalizing on LC-MS to simultaneously and specifically detect multiple molecular species, it is used to detect multiple peptides generated following enzymatic digestion of proteins in biological matrices by SRM and accurate mass on high resolution mass spectrometers. There are advantages in the simultaneous detection of multiple peptides in samples from preliminary experiments that provide additional information which is not always possible by LBA. By utilizing immunocapture procedures in sample preparation and analysis by low-flow chromatographic systems coupled with novel interfaces, rapid develop of very sensitive methods and integration into targeted protein analysis in biological fluids early in the discovery environment is becoming more prevalent.

Keywords: Pharmaceutical, Immunoassay, Liquid Chromatography/Mass Spectroscopy
Application Code: Drug Discovery
Methodology Code: Liquid Chromatography/Mass Spectrometry
The benefits of microflow in ESI mass spectrometry are well documented in the literature and interest in microflow LC-MS/MS is growing rapidly in routine laboratories. The true benefits of microflows LC/MS can only be realized in solution embodiments where every component of the fluidics is optimized for micro or capillary flow. This talk will discuss several different implementations of microflow LC for quantitation in various biological matrices with robustness of 2.1mm column workflows. Examples showing robust microflow LC-MS/MS operation that deliver benefits close to theoretically expected gains will be presented. These include quantitation of peptides such as Exenatide in plasma, endogenous steroids in dry blood spots, pain panel screening in urine and others.

Keywords: Bioanalytical, Capillary LC, Liquid Chromatography/Mass Spectroscopy, Quantitative
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
New Technologies and Methods in Protein Quantitation for Biotherapeutics and Clinical Diagnostics

Next Generation Plasma Collection Technology for Clinical and Pharmaceutical Applications

Mass spectrometry (MS) has become a major tool in drug discovery, clinical diagnostics, and personalized medicine. With the rapid progress being made in various modes of mass analysis, MS detection sensitivity, and analyte quantification, it is surprising that the accompanying blood collection and sample preparation technologies are so antiquated. Acquisition of blood by venipuncture goes back hundreds of years, while the dried blood spot approach of Guthrie is now a half century old. The work reported in this presentation will focus on a new technology that exploits the benefits of collection, drying, and transporting small samples on paper, but after the removal of blood cells and collection of a fixed volume of plasma. Subsequent to deposition of a blood drop on a small card composed of a laminated membrane stack, the sample rapidly spreads laterally by capillary action in the first membrane layer and then proceeds as a front into a second membrane where cells are removed by a combination of adsorption and filtration as plasma is drawn down through the membrane matrix. Migration of plasma through the membrane system terminates in roughly 3 minutes with the collection of either 2.4 or 4.8 μL of plasma in a collection disc at the bottom of the membrane stack. The volume of sample collected between cards varied less than 2% over a hematocrit range from 20% to 71%. At this point the cell bearing upper layers of the membrane assembly were stripped from the card, exposing the plasma filled collection disc to the atmosphere. Within 15 min of air exposure the plasma loaded disc was sufficiently dry to be placed in an envelope for transport by mail or air courier. In both metabolomics and proteomic analysis, plasma derived from the plasma extraction card was comparable to that obtained by conventional venipuncture methods.

Keywords: Biological Samples, Clinical Chemistry, Liquid Chromatography/Mass Spectroscopy, Sample Preparation

Application Code: Bioanalytical

Methodology Code: Sampling and Sample Preparation
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. To determine the utility of micro flow LC-MS/MS for routine bioanalysis of large protein therapeutics and biomarkers, a method was developed and validated for the quantitative analysis of Human monoamine oxidase B (MAO-B) in plasma.

Procedures and Equipment
Organic precipitation and trypsin digestion were used to prepare samples of human MAO-B in plasma for analysis by MFLC-MS/MS. An Eksigent Express HT-Ultra LC® system coupled with an ABSCIEX 5500® QTRAP mass spectrometer was used for MFLC/MS/MS analysis.

Results
Tryptic digestions of MAO-B provided signature peptides for MFLC/MS/MS analysis. A stable-labeled peptide internal standard was utilized for quantitation. Sensitivity, selectivity, accuracy, and precision were acceptable for routine bioanalysis.

Conclusion
Here we have demonstrated a validated, high throughput method for large molecule bioanalysis using MFLC-MS/MS. This data, along with the sensitivity and efficiency benefits of MFLC-MS/MS, suggests that the technique has value as a bioanalytical technique for large molecule therapeutics and biomarkers.

Keywords: Bioanalytical, Liquid Chromatography/Mass Spectroscopy, Protein, Tandem Mass Spec
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Absolute quantification of peptides, which serve as surrogate analytes for their representative proteins, places unique demands on the throughput, chemical specificity and selectivity of LC-MS/MS. The nanospray (low flow) variant of electrospray ionization has been the method of choice for qualitative peptide analysis by mass spectrometry. It is emerging as a method of choice for peptide quantification.

Nanospray is typically employed in two very distinct, almost unrelated, forms. Off-line, or static nanospray, is characterized by ultra-low flow rates (< 20 nL/min). The second implementation of nanospray, on-line nanospray, is combined with nanobore LC. It is characterized by controlled and pumped flow rates of 200-1000 nL/min. Unfortunately much of the significant analytical benefit of nanospray (efficiency, ionization response, matrix effect reduction) is lost even at “modest” low flow rates of 100-200 nL/min. On-line nanospray enables highly complex mixture analysis, and reasonable throughput through a traditional, though limited, LC approach. Analytically, the analyst would benefit greatly from a new methodology for nanospray combining these two worlds. Two novel approaches for combination of these two methods will be presented. The first is a highly miniaturized and optimized form of solid phase extraction (SPE) combined with off-line nanospray. The second approach is the adoption of segmented flow microfluidics to combine high throughput with on-line sample handling. Both enable high throughput, ultra-low flow nanospray workflow suitable for many areas of chemical analysis and quantification by mass spectrometry.

Keywords: Capillary LC, Liquid Chromatography, Mass Spectrometry, Proteomics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Mass Spectrometry
Dendrimer Based Terahertz Spectroscopy Applications With Examples in Fullerenes and Single Nucleotide Polymorphism

Dendrimer is a polymeric nanomaterial with spherical molecular architecture. An electro-optic (EO) dendrimer allows generation of high power, CW terahertz radiation (T-Ray) via dendrimer dipole excitation [1]. We utilized an EO dendrimer source to produce a terahertz time-domain spectrometer with very high sensitivity over a wide THz range. Unlike other spectrometers, e.g., IR, Raman, NMR, that can probe only motions associated with a bond, T-Ray is sensitive to different kinds of resonances present in a molecule, such as vibrational, translational, rotational, torsional, conformational, etc. Two important factors for effective characterization of molecular phenomena are the sensitivity and the penetration depth. The better the sensitivity, the smaller the sample requirement; and the deeper the penetration, the richer the information that can be extracted. Additionally, T-Ray is non-ionizing; consequently, probing is highly intrinsic without perturbation. From these considerations, T-Ray has the right kind of properties for information-rich spectroscopy applications. A terahertz spectrometer is comprised essentially of a broadband THz emitter, and a detection system composed of the same material as the emitter. Measurement is made in the time-domain, over a range of sub-pico seconds to a few tens of pico-seconds. The time-domain signal is analyzed via Fourier transform to produce the spectrum. With a 35 THz observation window, we have established that “no two molecules are the same;” because the THz spectra of any two molecules are distinctly distinguishable. In this paper, we describe examples of detecting hydrogen and deuterium impregnated Fullerenes and single nucleotide polymorphism (SNP) by terahertz spectrometry. SNPs are DNA sequence variations that occur when a single nucleotide (i.e., A, T, C, or G) in the genome sequence is altered. SNPs make up about 90% of all human genetic variation. Label-free SNP detection is of fundamental importance in the identification of genetic and hereditary diseases and also for the development of personalized medicine. A specific gene sequence has been investigated to show that when a G is replaced by a T, the change is clearly exhibited by their characteristic spectra.

Originated in the Russian literature in the 1990s, the concept of radar detection of radioactive clouds has intrigued many researchers due to its novelty and potential for remote nuclear detection from far greater distances than possible with conventional detectors. Categorizing the nuclear radiation-induced ionization effects in air as a time progression of plasma, water clustering, and microdroplet formation, Argonne has conducted a systematic study - theoretically and experimentally with nuclear sources - of radar cross section of ionized air targets in the RF to microwave to millimeter wave spectral bands. This talk will present an overview of the theory and results of electromagnetic detection of nuclear radiation and materials.

Keywords: Detection, Microwave, Plasma
Application Code: Nuclear
Methodology Code: Sensors
For wafer inspection purposes a terahertz 3D nano-imager has been designed and fabricated at Applied Research & Photonics, Inc. A simultaneous reflection and transmission measurement allows inspection of semiconductor wafers during fab processes (in-situ) as well as post-fab characterizations (ex-situ). For reflection measurements, the reflected intensity is proportional to the physical properties of the specimen under test, such as the refractive index, density, surface texture, dielectric constant, etc. For a given wafer, all material parameters remain unaffected during measurements, because terahertz radiation is non-ionizing and thus do not perturb the intrinsic properties. The reflectance, in this case, is a function of different material properties on the substrate. That is, the reflectance, R, is proportional to the variation in material conditions; thus, measurement of \( R(x, y, z) \) will yield the characteristics of the features (patterns) on the substrate. In addition, if there is a hole or void on the substrate or in any of the sub-surface layers, that will show in both reflected and in transmitted intensity. Based on the above principle, a signature of a given defect may be established. Any defects such as, inclusions, cracks, non-uniformity, or particulate foreign material can be detected and identified by this technique. Any phase separation between two adjacent materials and/or delamination between successive layers can also be detected. Moreover, defect size may be estimated from a reconstructed 3-D scan. While some optical scanners can examine the surfaces, a terahertz scanner is able to penetrate and interrogate sub-surface layers in a non-destructive fashion. The terahertz nano-scanner deploys a non-contact measurement system with an adjustable stand-off distance. It is adjustable to accommodate many sample sizes, e.g., a 450 mm wafer. A rotary axis enables examination of the sample from different viewing angles. This is important because cracks or other non-uniformity might NOT be along a straight line-of-sight. Thus an angular scan enables viewing hidden features. Details will be discussed with practical data.

Keywords: Imaging, Instrumentation, Sensors, Surface Analysis
Application Code: Other (Specify)
Methodology Code: Other (Specify)
The remote measurement of physiological signals is the most desirable scenario when sensing must be performed in a noninvasive or covert manner. The more prominent applications include healthcare, for monitoring of patients; first response, for locating of buried subjects; and national and homeland security, for standoff biometric screening. Microwave energy can penetrate through many optically opaque dielectric materials, such as common fabrics, with relatively low levels of attenuation and in turn allow for probing of surface reflections from underlying biological matter. This ability renders this electromagnetic frequency range particularly useful for noninvasive measurement of physiological movements. The use of microwave techniques for remote monitoring of human vital signs has been investigated by a number of researchers in the past. The majority of work in this area has been conducted within the microwave band, which is commonly designated as the frequency range between 300 MHz and 30 GHz, with most of the devices operating at the lower end of the microwave frequency range. As a direct consequence of operating at shorter wavelengths, millimeter-wave (MMW) frequencies, which are commonly designated as the frequency range between 30 and 300 GHz, provide higher sensitivity than their microwave counterparts for the detection of physiological movements. Fewer studies, however, have been reported on the use of MMW technology for remote measurement of human vital signs. Investigations conducted at the upper end of the MMW range, on the other hand, have employed quasi-optical techniques using table-top systems composed of high-frequency solid-state components and laboratory instruments. An overview is presented of the more recent advances in remote sensing of physiological signals using microwave and millimeter-wave technology, with emphasis on biometric sensing applications.

Keywords: Biomedical, Detection, Microwave, Sensors
Application Code: Other (Specify)
Methodology Code: Sensors
In recent past, much effort has been expended on developing high-resolution, (near) real-time, portable, and ultimately 3D microwave and millimeter wave imaging methods. There are several different design approaches that may be utilized for his purpose. However, for nondestructive testing and evaluation (NDT&E) applications there are certain additional constraints that must be accounted for when designing such systems in addition to constraints set by microwave engineering and electromagnetic principles and limitations. Design and overall optimization of such unique systems from beginning to end includes: 1) the design of an antenna that may be used singularly (when raster scanning) or in a collector array format, 2) data collection strategies, 3) imaging algorithm design, and finally 4) rendering of the data. This presentation gives an overview of the recent activities in this area at the Applied Microwave Nondestructive Testing Laboratory (amntl), Missouri University of Science and Technology (S&T), showing the progression of advancements in this field towards achieving real-time 3D microwave and millimeter wave imaging systems. Utility of different antenna designs as well as data collection methods for this purpose will be discussed.

Keywords: Imaging, Microwave
Application Code: Other (Specify)
Methodology Code: Sensors
Doppler radars have broad applications like velocity monitoring of cars, human tracking, building vibration monitoring, and even human heart rate non-contact sensing. Conventional Doppler radars at microwave and millimeter wave usually employ expensive mixers. Even worse, such mixers are still not available at the THz regime. To overcome these shortcomings, we invented our patent-pending low-cost, mixerless Doppler radar based on interferometry technique. The universal, mixerless millimeter wave (mmW) and THz wave Doppler radar architecture consists of simply a Continuous Wave (CW) source and an intensity detector based on optical interferometry technique has been assembled. The phase information is obtained by using an oscillating mirror in the reference arm, similar to that used by the FTIR (Fourier Transform Infrared spectroscopy) technique. The reference mirror oscillates at a frequency that is higher than twice the Doppler frequency of the object. Rigorous mathematical formulas have been derived to solve for both the amplitude and the phase of the Doppler signal, by using the Low-Frequency-Band (LFB) and High-Frequency-Band (HFB) signals. The Doppler frequency signature of a moving object can be obtained from the Fourier transform of the phase. Two prototypes at 94 GHz and 0.15 THz were built and tested using a ball pendulum target moving over a full-swing distance much smaller than a wavelength. Both the measured amplitude and phase have been shown to agree well with the experimental parameters. Extension to THz regime is straightforward and will be discussed.

**Abstract Text**

Doppler radars have broad applications like velocity monitoring of cars, human tracking, building vibration monitoring, and even human heart rate non-contact sensing. Conventional Doppler radars at microwave and millimeter wave usually employ expensive mixers. Even worse, such mixers are still not available at the THz regime. To overcome these shortcomings, we invented our patent-pending low-cost, mixerless Doppler radar based on interferometry technique. The universal, mixerless millimeter wave (mmW) and THz wave Doppler radar architecture consists of simply a Continuous Wave (CW) source and an intensity detector based on optical interferometry technique has been assembled. The phase information is obtained by using an oscillating mirror in the reference arm, similar to that used by the FTIR (Fourier Transform Infrared spectroscopy) technique. The reference mirror oscillates at a frequency that is higher than twice the Doppler frequency of the object. Rigorous mathematical formulas have been derived to solve for both the amplitude and the phase of the Doppler signal, by using the Low-Frequency-Band (LFB) and High-Frequency-Band (HFB) signals. The Doppler frequency signature of a moving object can be obtained from the Fourier transform of the phase. Two prototypes at 94 GHz and 0.15 THz were built and tested using a ball pendulum target moving over a full-swing distance much smaller than a wavelength. Both the measured amplitude and phase have been shown to agree well with the experimental parameters. Extension to THz regime is straightforward and will be discussed.

**Keywords:** Biomedical, Integrated Sensor Systems, Microwave, Sensors

**Application Code:** General Interest

**Methodology Code:** Sensors
Microwave and millimeter wave techniques are relative newcomers to the field of nondestructive testing and evaluation (NDT&E), utilizing electromagnetic waves in the frequency range of ~300 MHz-300 GHz. In the distant past, attempts in using these techniques for NDT&E purposes were limited and they were referred to as “emerging techniques.” However, those involved in the science and engineering of microwave materials characterization, radar remote sensing, and microwave system development have always recognized the utility of these techniques for NDT&E applications. There are several prominent factors that have helped bring these techniques to the forefront, namely: the increasing utility of composite structures, availability of large signal bandwidth, plurality of ways by which these waves interact with material media, availability of numerous testing probes, and increased availability of critical devices and components. These techniques have a wide range of applicability to NDT&E, namely; for materials characterization, evaluation of a host of critical parameters in cement-based materials, surface-breaking crack detection in metals and dimensional evaluation, comprehensive evaluation of layered composite structures, high-resolution imaging for flaw detection, etc. This presentation gives an overview of the utility of these techniques as well as several key examples of applications.
Most semiconductor based THz detectors and emitters have dimensions that are far smaller than the THz wavelength, and therefore, they need optical antennae to efficiently couple the THz radiation from far-field to the sub-wavelength device. We have developed a novel metallo-dielectric antenna with very large antenna gain that is less than 3dB below the maximum theoretical limit when the detector/emitter volume is orders of magnitude smaller than the wavelength cubed. The power coupling of our antenna is more than an order of magnitude better than a bow-tie antenna to far field.

Also, we have developed a novel field-induced tunable quantum dot that is able to detect THz radiation selectively over a wide tuning range. Unlike the conventional quantum dots, the electron confinement in our device is not based on the geometry of the device, but rather is induced with an external electric field. This feature allows ultra-fats tuning of the wavelength over a large bandwidth. More importantly, an imager built with such elements could tune individual pixels independently to produce adaptive and dynamic spectral imaging.

Keywords: Array Detectors, Biosensors, Detector, Semiconductor
Application Code: Pharmaceutical
Methodology Code: Sensors
2-pyridinedicarboxylic acid (DPA), a substance uniquely present in bacterial spores, is an important useful biomarker in the detection of anthrax spores. In this work, a sensitive method for the detection of DPA is developed by using an engineered biological protein ion channel in the presence of a molecular probe. This method employs single-channel recording with the planar lipid bilayer technique, where current modulation represents individual binding events. In the absence of DPA, the molecular probe only produces a major type of events. However, in the presence of DPA, a new type of current blockage events with significantly different residence times and amplitudes is identified, and the concentration of DPA could be related to the frequency of the new events. The method could detect DPA at nanomolar levels, and should find a potential application as a low-cost screening sensor to monitor anthrax in powder and liquid samples.
Application of Bioanalytical Sensors

Enhanced Stability of Suspended Lipid Bilayers for Ion Channel Recordings and Biosensor Development

Black lipid membranes (BLMs), are synthetic membranes used to measure ion channel activity. The development of next-generation transmembrane protein-based biosensors relies heavily on the use of BLMs. BLMs instability resulting in rupture within hours (< 4 h) of formation poses a significant challenge to biosensor development. Enhanced temporal, mechanical, and electrical stability of BLMs is needed to support the development of membrane-based biosensors. Here, we employed chemically modified aperture surfaces and chemical cross-linking within the lipid membrane to dramatically improve BLM stability. Glass microapertures were modified using tridecafluoro 1, 1, 2, 2-tetrahydrodimethylchlorosilane. The amphiphobic property (H2O/oil repellency) of the perfluorinated surfaces facilitated the rapid formation of highly stable BLMs. Perfluorinated patch pipettes showed decreased background capacitance and ionic conduction by 83% and 77% respectively, compared to unmodified pipettes. The reduced background current led to 48% noise reduction compared to conventional pipettes. BLM stability was further improved by photopolymerization of ethylene glycol dimethacrylate and butyl methacrylate partitioned into 1, 2-diphytanoyl-Sn-glycero-3- phosphocholine lipid membranes. Overall, cross-linked BLMs suspended on perfluorinated apertures exhibited significantly improved lifetimes (> 24 h), 25-fold increase in mechanical stability, 50% increase in electrical resistance of BLMs, and 53% increase in electrical stability. Highly stabilized BLMs will play an important role in the development of membrane-based biosensors.

Abstract Text

Bioanalytical, Biosensors

Application Code:  Bioanalytical

Methodology Code:  Sensors
Label-free optical sensing has gained considerable attention for applications such as protein and small molecule/drug analysis, environmental monitoring, and in vitro assays. However, extracting both qualitative and quantitative information from a sensor surface has been difficult due to lack of a suitable technical platform and an inability to distinguish nonspecific binding within a complex sample. Our research focuses on developing a nanomaterial-based, label-free detection platform that offers the ability to couple multiple types of assays, allowing for cross-platform measurements of molecules on a single biochip. To accomplish this, we have functionalized gold nanoparticles (AuNPs) of varied shapes and sizes, and constructed ultrathin films in a layer-by-layer self-assembly process on a glass substrate. Our final product is a silica-coated AuNP film that is biocompatible, robust, and exhibits attractive plasmonic properties. The LSPR phenomenon has allowed for label-free optical detection; in addition, surface-enhanced Raman spectroscopic measurements can be achieved. Furthermore, the calcination process allows for laser desorption/ionization (LDI) mass spectrometry to be performed directly on the substrate without a normally required organic matrix. The combined detection, identification, and characterization performed on our AuNP film make it a unique and powerful new tool to meet future bioanalytical challenges.

Keywords: Biosensors, Mass Spectrometry, Materials Science, Spectroscopy
Application Code: Bioanalytical
Methodology Code: Sensors
## Abstract

Solid, porous materials are widely used in chemical and biomedical applications including chemical separations, drug delivery and catalysis due to their unique properties such as high surface area, ease of surface derivatization and interconnected pore structure. The functional properties of these materials are determined by solute interactions at the solid interface while being confined in nanometer-sized pores. For hydrophobically-derivatized nanoporous silica, the pores need to be wetted in order for an aqueous solution to carry the solute molecules into the pores. In this project, we employ surfactant molecules to induce wetting of the hydrophobic nanopores. With the use of fluorescent probes, not only can surfactant-induced wetting of the nanopores be monitored but they can also help elucidate the interactions at the solute-solid interface during the wetting process. A better understanding of the molecular behaviors confined at the nanoscale will enhance the effectiveness of porous materials in numerous fields and applications.

### Keywords
- Fluorescence
- Modified Silica
- Surfactants

### Application Code
- Bioanalytical

### Methodology Code
- Fluorescence/Luminescence
Application of Bioanalytical Sensors

Nanopore Stochastic Sensing of HIV-1 Protease

The human immunodeficiency virus type 1 (HIV-1) protease is a retroviral aspartyl protease, and has been recognized as the essential element in maturation of the infectious virus. Accordingly, HIV-1 protease has been an important target for drug therapy. Here, we report a rapid, sensitive, and label-free nanopore sensing method for the detection of HIV-1 protease, in which an engineered alpha-hemolysin is utilized as the sensing element, while an unmodified peptide is used as the substrate. Under a fixed potential bias applied across the nanopore membrane, the activity of the HIV-1 protease can be detected and quantified by monitoring the change in the ionic current passing through the nanopore, which is due to the cleavage of the peptide by the HIV-1 protease. The method is sensitive (can detect HIV-1 protease at pico- to nanomolar levels) and selective (other proteases will not interfere with the detection). The substrate-based nanopore sensing approach should find useful application in the development of nanopore sensors for other proteolytic enzymes.

Keywords: Bioanalytical, Biosensors, Nanotechnology, Sensors

Application Code: Bioanalytical

Methodology Code: Sensors
Application of Bioanalytical Sensors

Signal Amplification Strategies on Nucleic Acid-Based Lateral Flow Biosensors

Traditional lateral flow biosensors are based on gold nanoparticle labels, the low sensitivity of the biosensors limits its applications. Recently we developed various strategies to enhance/amplify the signals of the biosensors. Enzyme/gold nanoparticle dual labels, carbon nanotubes and silica nanorods have been used as labels for the signal amplifications. The detection limits of the biosensor were lowered 50 to 100 times compared with the traditional gold nanoparticle-based lateral flow biosensors. It shows great promise for the detection of extremely low concentrations of analytes, such as protein biomarkers and nucleic acids.

Keywords: Biosensors, Nanotechnology, Sensors
Application Code: Bioanalytical
Methodology Code: Integrated Sensor Systems
In blood coagulation, the interactions of soluble proteins with cell membranes and membrane-bound proteins play a critical regulatory role, and lipid composition greatly mediates these interactions. A better understanding of lipid effects could lead to new pharmaceuticals to treat bleeding related conditions such as hemophilia. By creating arrays of phospholipid bilayer Nanodiscs on a silicon photonic microring resonators platform, we are able to directly probe these interactions in a label-free and multiplexed assay format. Nanodiscs are native-like lipid bilayers contained within an amphipathic protein and allow control over lipid composition. Silicon photonic microring resonators are a highly multiplexable sensor technology that allows for the real-time monitoring binding interactions without the need for fluorescent or enzymatic labels. Previous work has shown that interfacing nanodiscs with microring resonators can be used to study protein-lipid and protein-membrane bound protein interactions. This approach has been recently extended to probe the binding of proteins important to blood clotting (Factor VIIa (FVIIA), Factor IX (FIX), Factor X (FX), and Activated Protein C (APC)) to nanodiscs containing varying ratios of phospholipids containing phosphatidylcholine, phosphatidylserine, and phosphatidic acid. These experiments enable the determination of protein binding stoichiometry as well as the kinetic rate constants that govern binding and unbinding. We also have studies the synergistic effects of lipid composition as they regulate these interaction in a non-linear manner. Future extensions of these combined technologies will focus on the influence of more complex lipid compositions as well as the first ever investigations of in situ generated multi-protein complexes that are incredibly important regulators of the blood coagulation cascade.

This research was supported by the University of Illinois at Urbana-Champaign and the Alfred P. Sloan Foundation.
Implanted medical devices (IMDs) offer many benefits to the patients, however, they are susceptible to bacterial colonization and infections. These infections are difficult to treat as the bacteria form biofilms on the implant surface, which reduces antibiotics penetration and generates dormant regions. Additionally, early bacterial infection diagnosis is difficult as the bacteria are often localized on inaccessible regions on the implant surface. Herein, a radioluminescent sensor film is developed to map local acidosis on an implanted surface. The sensor films comprise both X-ray radioluminescence particles (gadolinium oxysulfide doped with europium ions) which serve as a light source and a pH indicator (bromophenol blue) that modulates the luminescence spectrum. When irradiated with an X-ray source, the particles emit red and near infrared emissions with good tissue penetration depth and negligible autofluorescence background from biological systems. Meanwhile, the basic form of the pH indicator absorbs more of the red luminescence peak than the near infrared one and subsequently the spectral ratio indicates pH. With a collimated X-ray beam (1 mm) as a point excitation source, the spatial resolution of the sensor film is largely improved. Calibration curves with or without passing through porcine tissue are generated. Furthermore, the bacterial growth caused pH acidity on the sensor film surface is mapped through porcine tissue. The proof-of-principle here demonstrated that it is feasible to noninvasively detect bacterial infection on IMD surface in vivo with high sensitivity and good spatial resolution. Acknowledgements: This research was supported in part by SCbiomat Center of Biomedical Research Excellence (COBRE) through NIH award 1R15EB014560.
### Abstract Text

Surface plasmon resonance (SPR) biosensing does not allow differentiation between a specific recognition event such as an enzyme-antibody binding and nonspecific adsorption (NSA) of the constituents of a complex matrix. For this reason, nonspecific fouling of the biosensor surface must be reduced to a minimum to allow sensitive and reproducible biodetection of analytes in a complex matrix. Cell lysate is an attractive biological matrix, as it provides access to analytes found in higher concentration in solid tissues or tumors. Human embryonic kidney (HEK293) cells were used to produce cell lysate, as they provide a close model to human cells. Several physico-chemical properties of the 3-MPA-XmYnZp OH peptides (where X,Y and Z are amino acids and m,n,p = 0-6) monolayer, including charge, polarity and hydrophobicity, were investigated to reduce NSA. It was found that hydrophobic and positively charged monolayers were more efficient at reducing NSA of cell lysate. For example, a peptide composed of 5 serine residues (contact angle of 21°, hydrophilic and negatively charged) had a surface coverage of 1156 ± 141 ng/cm2, in opposition to the 3-MPA-(His)2(Leu)2(Phe)2-OH peptide (contact angle of 77°, hydrophobic and slightly positively charged) with a surface coverage as low as 159 ± 27 ng/cm2 in a highly concentrated cell lysate. All the results obtained in this study follow the same trend and provide a better understanding of the processes occurring during NSA. To further comprehend these mechanisms, Matrix-Assisted Laser Desorption Ionization/Time of Flight Mass Spectrometry identified the molecules bound to various surfaces, such as PC(16:0/18:1), a common lipid found in human cells. This study confirmed that the interactions between the matrix and the surface are completely different that the ones occurring in bovine serum; thus screening a novel series of SAM was necessary to develop a proper surface chemistry for bioanalysis in crude cell lysate.

### Keywords
- Biosensors
- Biospectroscopy
- Peptides
- Surface Analysis
These studies describe the implementation of second harmonic correlation spectroscopy (SHCS) to measure the adsorption and desorption kinetics of molecular species associating with a surface. Specifically, the local fluctuations of the measured second harmonic (SH) signal were used to determine the binding kinetics and thermodynamics of small molecule and protein – ligand association. First, the investigation of a small molecule, (S)-(+)-1,1'-bi-2-naphthol SBN, intercalating into a 1,2-dioleoyl-sn-glycero-3-phosphocoline (DOPC) bilayer demonstrates that SHCS can be used to provide accurate kinetic and thermodynamic binding data for molecules at a surface. The sensitivity of SHCS was further probed by examining more complex systems, such as small molecule association to a lipid bound polypeptide receptor and multivalent protein binding to a ligand doped lipid bilayer. SHCS reduces both the amount of analyte required and collection time as compared to traditional binding isotherm studies. Additionally, the surface specificity and label-free nature of SHCS eliminate some of the challenges seen in fluorescence correlation spectroscopy. The simplicity and efficiency of SHCS makes it a new and valuable technique to directly and precisely ascertain the binding kinetics of molecules at a surface without a label.

Keywords: Absorption, Bioanalytical, Biospectroscopy, Protein
Application Code: Bioanalytical
Methodology Code: Biospectroscopy
Gold nanoparticles have been extensively used as drug or gene delivery vehicles into biological cells for targeted imaging and therapeutic applications. How surface-functionalized gold nanoparticles interact with cell membrane upon initial contact is still unclear. In this study, we investigate the rotation dynamics of surface-functionalized gold nanorods rotation at the early stage of their binding to cell membrane. The gold nanorods are excited at their longitudinal surface plasmon resonance (LSPR) and collected using a confocal-type setup. Such a resonance light scattering setup allows us to see particle rotation with a temporal resolution of 500 ns. The resonance scattering photon bursts are analyzed with correlation spectroscopy. The experiments show that on negatively charged glass surface, the rotation of the positively charged 25 x 74 nm nanorods slow down significantly when they are loosely adsorbed on glass surface through electrostatic interaction. The characteristic rotation time increases from 30 microseconds to ~ 1 ms. Intermittent slow rotation dynamics with a characteristic time of several hundred ms can be observed. On cell membrane, similar rotation pattern can be observed, indicating the initial nanorod-membrane interaction is dominated by electrostatic interaction. However, the rotation dynamics slows down gradually, indicating the particle is interaction with the membrane with other types of forces. The final characteristic rotation time of several hundred ms possibly suggests that the entire patch of the membrane underneath the nanorod is moving together with the nanorod. This study sheds new light on how gold nanoparticles interact with cell membrane.
Atomic Force Microscopy (AFM) is a powerful tool for studying biomolecular systems due to its high spatial and force resolution capabilities. Exploring small forces associated with biological processes such as enzymatic motion during catalysis or ligand-substrate binding is important for understanding biomolecules on a fundamental basis. The current study utilizes Molecular Recognition Force Spectroscopy (MR-FS) to study an enzyme binding a drug molecule. The drug decorated AFM probe approaches the enzyme bound surface, the molecules bind, and the adhesion force between the two is measured. This method can also elucidate kinetic details of the binding/unbinding processes. However, many non-specific interactions are similar in magnitude to the adhesion force of interest, consequently lowering the accuracy of the measurement. Linking molecules such as polymers or DNA are often times used to tether the receptor to the sample surface or the ligand to the probe and can be used as a means to distinguish non-specific binding events that occur when the probe is in close proximity to the sample surface from those of the specific binding event. This study explores the effect of a double-strand DNA linker to tether the enzyme dihydrofolate reductase (DHFR) to the sample surface and a polyethylene glycol (PEG) linker to attach the drug methotrexate to the AFM probe. The data suggest that the use of linkers provides more accurate force measurement by creating a way to distinguish and eliminate non-specific interaction forces from the data and also increasing the likeliness of single-molecule interactions.

Keywords: Bioanalytical, Biological Samples, Biospectroscopy, Immobilization
Application Code: Bioanalytical
Methodology Code: Biospectroscopy
New pattern recognition techniques have been developed to search IR spectral libraries of the PDQ automotive database to differentiate between similar but nonidentical IR paint spectra and to determine the model and line of the vehicle from a paint sample recovered at a crime scene. Currently, modern automotive paints use thinner undercoat and color coat layers protected by a thicker clear coat layer. As a result, only a clear coat paint smear is often the only layer of automotive paint left at a crime scene. In these cases, the PDQ database cannot identify the vehicle because of the reliance of the text based search on the large variations in color and chemical composition of the other automotive paint layers. To assess the evidential information content of a clear coat paint smear, pattern recognition techniques have been developed to search IR spectral libraries of the PDQ database. Spectral search algorithms commercially available cannot distinguish the subtle differences between clear coat paint spectra from one vehicle model to the next. To tackle the problem of library searching in the PDQ database, a prototype library search system to identify the assembly plant, model, and line of an automobile from its clear coat paint spectrum has been developed. The system consists of two separate but interrelated components: search prefilters to cull the library spectra to a specific assembly plant or set of plants and a cross correlation searching algorithm to identify spectra most similar to the unknown in the set identified by the search prefilters. The library search algorithm, which cross correlates an unknown with each IR spectrum in the set identified by the search prefilters, is sufficiently sensitive at distinguishing subtle but significant features in these spectra, e.g., shoulders and minor peaks, which may be highly informative but are ignored when using commercial library search algorithms.

Keywords: Molecular Spectroscopy, Paint/Coatings, Statistical Data Analysis, Vibrational Spectroscopy
Application Code: Homeland Security/Forensics
Methodology Code: Chemometrics
Releases of the high-impact greenhouse gas CO2 into the atmosphere has become a serious concern. On the other hand, half of the global primary carbon production is due to algal photosynthesis consuming bicarbonate produced from atmospheric CO2. Therefore, understanding how phytoplankton transformations inorganic compounds into biomass is of relevance for environmental chemistry in particular for building predictive models simulating future environmental scenarios.

This sequestration of inorganic compounds is complicated by algae requiring a mix of nutrients and by species competition for these nutrients. Ongoing research is linking —statically— nutrient availability to the resulting microalgal biomass. However, investigating the dynamics of such multi-nutrient sequestration processes requires novel analytical approaches for monitoring live cells. Experimental data presented here have been acquired by means of FTIR-ATR. While living cells slowly form a biofilm on an ATR crystal, their changing spectroscopic signature can be probed every few minutes. The information about the dynamics of their chemical adaptation is not directly accessible though because two processes contribute to the change in spectroscopic signatures, i.e. cell accumulation and environment-induced chemical changes. To separate these two effects, pairs of time series of FTIR-ATR spectra are acquired from two different nutrient conditions. This then enables a discrimination of the physical dynamics (sample accumulation) and chemical dynamics (change in the cells’ composition). Experimental results obtained from concentration dependent impacts of bicarbonate, ammonium, and nitrate on the chemical composition of Dunaliella parva will be presented. It is anticipated that these analytical tools will then enable simulations of different environmental scenarios for prediction of phytoplankton’s sequestration of anthropogenic compounds released into marine ecosystems.

Keywords: Biological Samples, Chemometrics, Environmental/Water, FTIR
Application Code: Environmental
Methodology Code: Computers, Modeling and Simulation
Near infra-red (NIR) spectroscopy is widely used in the pharmaceutical industry to identify raw materials and to monitor upstream steps for biopharmaceutical manufacturing. There are also numerous process control applications of NIR in the chemistry and pharmaceutical industries. Despite the acceptance of NIR as a specific, rugged spectroscopic technique, there remain hurdles to more wide-spread adoption in biologics manufacturing and research. The primary limitation is the challenge to interpret a NIR spectrum using chemical intuition. In industry it is often not enough to know that a process is out of trend; the reason needs to be identified.

This presentation describes a simple, effective way to combine NIR and nuclear magnetic resonance (NMR) by sequentially comparing NIR and NMR with partial least squares regression (PLS). Results provide a selection method for NIR wavelengths to include in a robust NIR-only model even before manufacturing process data is available. The technique is demonstrated using both simple and complex mixtures.

Keywords: Chemometrics, Informatics, Near Infrared, NMR
Application Code: Pharmaceutical
Methodology Code: Chemometrics
Comprehensive two-dimensional liquid chromatography (LC x LC) is achieved when the total effluent from one column is sequentially transferred into a second column. The number of transfers depends on the ratio between the first dimension analysis time and the first dimension sampling time ($t_{fs}$). We have previously recommended a $t_{fs}$ between 12 and 21 s for first dimension gradients ranging from 5 to 60 minutes. However, selecting $t_{fs}$ in this way only optimizes peak capacity but it does not ensure the best quantitation. Currently, a modulation ratio ($M_{R}$) is 3.0 recommended in the literature to ensure that the calculated two-dimensional peak area is resistant to fluctuations in the first dimension sampling phase ($\Phi_{1}$). However, these studies only examined the impact of $M_{R}$ and $\Phi_{1}$ from data at a single UV wavelength or from a TIC. In this talk, we will examine the impact of $M_{R}$, $\Phi_{1}$ and $t_{fs}$ on simulated fast on-line LC x LC multi-wavelength data using a PARAFAC (parallel factor) based method of peak quantitation. The PARAFAC method was essentially unaffected by small changes in $\Phi_{1}$ when $M_{R}$ was 0.5, 0.75, or 3.0. However, only when $M_{R}$ was at least 3.0 were the results relatively insensitive to larger variations in $\Phi_{1}$. Based on these results, we recommend that a $M_{R}$ of 3.0 be used when reproducible first dimension time cannot be achieved.
Chemometrics
Removing Correlation Degeneracies in Spectral Angle-Based Hyperspectral Image Analyses

Correlation methods including spectral angle mapping (SAM), cosine correlation analysis (CCA), and spectral identity mapping (SIM) are widely used to provide rapid chemical-based image contrast in hyperspectral image data. Because correlation methods measure the similarity between each member of the spectral set and a known reference spectrum, they are employed to identify unknown samples against a database of known spectra. For a large number of spectra, such as in hyperspectral image analyses, obtaining the same correlation score for two distinct spectra is probable and results in the same color (score) for different spectra.

In the work presented here, we have developed a protocol for removing the degeneracies caused by symmetry and mapping the results to the full 24-bit RGB color-space of modern computer displays. To evaluate this protocol’s effectiveness we have created hyperspectral image datasets with large numbers of spectra that exhibit symmetry about a given reference vector. These spectra, cast as spectral vectors in an orthogonal wavelength space, are well-distributed along the periphery of a hypercone having the reference spectrum as its axis. Applying this protocol effectively removes all symmetry in the dataset. 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. We compare results from real hyperspectral imaging data with and without implementing our algorithm. A concise protocol is put forth that will enable other researchers to utilize this method by following a short, simple list of steps.

Keywords: Chemometrics, Data Mining, Imaging, Raman
Application Code: Bioanalytical
Methodology Code: Chemometrics
Unique Ion Filter: A Strategy for GC-MS Data Processing Prior to Chemometric Analysis

GC-MS chromatograms collected with high scan rate (>10,000 amu/s) instruments are data-rich and often allow for the application of chemometric processing to the raw data. However, with the enormous volumes of data produced by these systems, the risk of inclusion of irrelevant and/or overly-redundant data in chemometric models cannot be over emphasized. Previously, we (and other researchers) have developed feature selection tools that may be applied to raw GC-MS data. However, when considering millions of individual data points for each chromatogram, these are computationally expensive. While multiple signals from the same compound contribute stability to the chemometric model and act together to reinforce each other, in our previous research it was noticed that several hundreds of data points would often be included for each molecule that was included in the model. Consequently we set to develop a filter that would automatically reduce the extreme redundancy in the data to a more moderate level without losing vital chemical information.

The concept that we introduce here is referred to as a unique ion filter (UIF). Consider a single GC-MS peak of width 6 s, scanned at 10 Hz, and an m/z range of 30-350, this peak is represented in the data set as 19,260 individual ion abundances. The concept of the UIF is to automatically select and reduce this to a few key features (<50) that still provide redundancy while greatly reducing the number of variables to be considered in subsequent feature selection and chemometric modeling. Preliminary results demonstrate that combining UIF and a previously developed feature selection algorithm leads to a significant improvement in computation time while generating classification models with comparable or improved separation between classes.

Keywords: Chemometrics, Forensics, GC-MS, Metabolomics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Chemometrics
The use of chromatographic profiles from volatile fractions of plant clones – in this case, hybrids of Eucalyptus grandis × Eucalyptus urophylla – to determine specimens susceptible to rust disease will be discussed. The analytes were isolated by headspace solid phase microextraction (HS-SPME) and analyzed by comprehensive two-dimensional gas chromatography combined to fast quadrupole mass spectrometry (GC×GC-qMS). Parallel Factor Analysis (PARAFAC) was employed to estimate the correlation between the chromatographic profiles and resistance against Eucalyptus rust, after preliminary variable selection performed by Fisher ratio analysis. The proposed method allowed the differentiation between susceptible and resistant clones and determination of resistance biomarkers. This approach can be a valuable alternative for the otherwise time-consuming and labor-intensive methods commonly used in plant pathology.
Environmental Analysis of Persistent and Toxic Compounds

Monitoring Endocrine Disruption in Japanese Medaka Fish Using Capillary Electrophoresis and Egg Hatching

Environmental exposure to endocrine disruptors can be monitored by determining the circulating steroids in fish plasma using capillary electrophoresis coupled to UV-visible absorbance detection. Profiling multiple steroids in a single sample is important in assessing the effects of endocrine disrupting chemicals on physiological regulation. Japanese Medaka fish are used as the animal model. The fish are paired and exposed to an endocrine disruptor for seven days. Circulating steroids are then determined using pH mediated stacking capillary electrophoresis that has quantification limits of 5 nM for absorbance detection. Individual fish are analyzed to simultaneously profile multiple circulating steroids. This yields more information about the effect of an endocrine disruptor on each fish as opposed to pooling the fish plasma, as is required in conventional methods of analysis. Endocrine disruption is also monitored by determining the amount of eggs produced and fertilized during a seven day exposure. The time it takes for the eggs to hatch is recorded and used to monitor the effect of an endocrine disruptor on a pair of mating fish. In this study it is demonstrated that the solvent vehicle used to expose the fish to an endocrine disruptor is essential. Certain solvents used to dose the fish are endocrine disrupting chemicals. The use of an endocrine disrupting solvent therefore complicates the toxicity study. Circulating levels of steroids determined with capillary electrophoresis are analyzed in addition to egg hatching data to obtain a more comprehensive toxicity study.

**Keywords:** Capillary Electrophoresis, Environmental Analysis

**Application Code:** Environmental

**Methodology Code:** Capillary Electrophoresis
Graphene oxide (GO)-based sensors have gained considerable attention recently as they offer a unique path to improve sensing performance. GO is a flat planer conjugated carbon ring nanostructure that facilitates pi interactions between polycyclic aromatic molecules and sp2 domains within the basal structure. This property allows for monitoring of emerging contaminants in complex environments such as PCBs and PBDEs. Here we report the development of new, inexpensive, and effective techniques with GO for environmental applications. GO ultra-thin films have been fabricated on silane-coated substrate and characterized with AFM, which shows well-defined assembly. The film allowed for Raman detection but the improvement was incremental. To further enhance the detection, we fabricated silver nanotriangles on the substrate using nanobeads and e-beam evaporation. Strong SERS has been observed from the nanotriangle-GO substrate and preliminary results with model compounds are very promising. We will extend the study to PCBs and other cytotoxic species in the environment. The surface will be optimized to increase sensitivity and robustness, and functionalized for effectively monitoring of environmental effluents. FDTD-based simulations have been carried out to provide guidance for optimization, and the results will be presented as well.
In recent years, comprehensive two-dimensional gas chromatography (GCxGC), a separation method recognized as offering far greater peak capacity than conventional one-dimensional separations, has been rapidly growing in popularity. A GC x GC separation is performed by interfacing a modulator between two columns, termed primary and secondary. The modulator is often referred to as the heart of the instrument. It functions to periodically sample, trap and inject the effluent from the first column into the second column. Today, the most frequently used GCxGC systems require consumables such as liquid N2 for the trapping function of the modulator. Although these systems are recognized as being very effective, their initial and running costs are a hindrance to more widespread use. A new, single-stage thermal modulator for GCxGC that requires no consumables for operation has been developed to overcome these problems. The device traps analytes through the use of a specially prepared coated stainless steel capillary compressed between two ceramic cooling pads. Analytes are thermally released from the trap into the secondary column via resistive heating. To evaluate this system, a routine accredited method for the analysis of polychlorinated biphenyls, organochlorine pesticides and chlorobenzenes was run using the new modulator and its performance was compared to that of an industry leading modulation system. Optimization aspects of the device design and performance comparison vs. a commercially available device will be presented.

Keywords: Environmental/Soils, GC, PCB’s, Quantitative
Application Code: Environmental
Methodology Code: Separation Sciences
Environmental Analysis of Persistent and Toxic Compounds

**Abstract Text**

Large scale fires including the Plastimet fire (Ontario) and The World Trade Centers (New York City) have brought into question the combustion products of brominated flame retardants (BFRs). Concern has arisen about the health effects experienced by first responders exposed to fire debris over long periods of time. In response, this study focuses on the investigation of the role mixed halogenated planar compounds, generated during the combustion of BFR-containing products, play in the toxicity experienced in first responders.

GCxGC was chosen as the analytical technique for characterization of fire debris samples due to advantages such as enhanced peak capacity and decoupled separation mechanisms. Therefore, isobaric compounds may be better resolved from one another, and more importantly from the matrix. In addition to coupling the GCxGC with time of flight mass spectrometry (TOFMS), high resolution TOFMS was also used to further distinguish between halogenated planar compounds and those with very similar nominal masses. High resolution TOFMS is also advantageous in this particular application due to lack of commercially available standards.

A GCxGC-TOFMS method has been developed for characterization of fire debris samples, with emphasis on identifying dibenzo-p-dioxins and dibenzofurans. Data from a simulated burn study show differing patterns of dioxins and furans depending on the type of fire (i.e. electronics rich vs. household simulation). An electronics rich fire generated a range of bromo/chloro dibenzofurans (PXDFs), as well as multiple bromo/chloro PAHs. Polybrominated dibenzofurans (PBDFs) dominated as the main halogenated planar combustion products in both the electronics rich and household simulation fire.

**Keywords:** Chromatography, Gas Chromatography, Gas Chromatography/Mass Spectrometry, Time of Flight MS

**Application Code:** Environmental

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Abstract Text

Instrument selectivity has always been an important factor for the gas chromatographic analysis of pesticide residues. Even the most sensitive detector can give poor detection limits when chemical noise interferes with the analysis. In the past, element-selective detectors such as the electron capture detector (ECD), flame photometric detector (FPD), and nitrogen phosphorus detector (NPD) were used to “see” various classes of pesticides in food extracts. These detectors were supplemented and then largely replaced by GC/MS in the scan or SIM modes. More recently, labs have converted to unit mass GC/MS/MS because of its very high selectivity and sensitivity, even for dirty QuEChERS extracts. A new instrument, the GC/Q-TOF potentially offers even greater selectivity with its accurate mass capability together with MS/MS. Even more selectivity should come from the use of chemical ionization techniques. This poster discusses the use of a GC/Q-TOF for the analysis of pesticide residues in food extracts with a focus on selectivity for pesticides over matrix components. Comparisons are made between single stage accurate mass TOF analysis and MS/MS experiments in the Q-TOF mode. Chemical ionization is considered for its ability to give higher abundances of high mass ions as precursors for MS/MS experiments.

Keywords: Environmental Analysis, Gas Chromatography/Mass Spectrometry, Pesticides
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Trace concentrations of pharmaceuticals in waste, surface and drinking water have been detected in a number of different locations. For example, the identification and quantification of chemotherapeutics in different stages of the water purification process has not been significantly reported, especially in the United States. This is likely due to the fact that a method focused towards targeting a variety of chemotherapeutic drugs in the initial and final stages of the wastewater treatment process has not been extensively studied. Although these agents share a common physiological effect, their structures and chemistries vary significantly. Thus developing a method that will allow efficient separation and detection of trace concentrations of these drugs is challenging. An additional issue with the analysis of these types of materials in a complex matrix is the very low-level of expected concentration. This places significant demands on both the sample preparation steps and also on the instrumental analysis.

The objective for this research is to develop a target-based analysis method that is capable of detecting a variety of chemotherapeutic agents at a level that is appropriate for the analysis of waste, surface and potentially drinking water. Due to the compounds lipophilic character a large-volume SPE-based sample preparation technique that is compatible with UHPLC analyses will be developed. In addition, the stationary phase column chemistries that provide the most efficient separation of a mixture of chemotherapeutic agents will be determined using UHPLC-PDA. Final determination of samples will be conducted using an UHPLC-triple quadrupole mass spectrometer to yield the necessary overall sensitivity and to allow for improvements in detectability.

Keywords: Drugs, Liquid Chromatography/Mass Spectroscopy, Solid Phase Extraction
Application Code: Environmental
Methodology Code: Liquid Chromatography/Mass Spectrometry
Abstract Text

Over the past few years, the QuEChERS method has grown in popularity due in part to its adaptability and increased efficiency for pesticide residues extraction and analysis. The general AOAC pesticide extraction method can be adapted to a wide range of products, spanning finished goods to their constituent agricultural products. The goal of the study was to determine if there were correlations between pesticide residues in the constituent agriculture products (grains, malt, hops, and grapes) and finished products (wine and beer) in the wine and beer-making process. Additionally, the study examined if there was an increase in pesticide recovery when the process was streamlined with automated sample preparation methods. The pesticide analysis methodologies are being pushed to increase the efficiency and recovery of the methods with an additional push towards optimization and automation of extraction and analysis methods for time and cost savings of analysis.

Keywords: Agricultural, Beverage, GC-MS, Pesticides
Application Code: Food Science
Methodology Code: Gas Chromatography/Mass Spectrometry
Current trends indicate that more than 500 compounds are routinely used under strict regulation on a global basis. With increasing global trade there is a requirement for multi-analyte screening strategies capable of efficiently detecting residue violations to protect consumer safety. Benefits of full spectra acquisition and the specificity of accurate mass measurement is well characterized and is used in combination with, time tolerances, isotope fits, fragment ions/ratios and response thresholds to reduce false positive/negative identifications in screening assays. Nonetheless, it is a challenge to identify targeted compounds present in the sample with a large number of co-extracted matrix components. The application of ion mobility to remove false positive identifications and importantly false negative identifications will be presented. The assay is based on the analysis of sample extracts and matrix matched calibrants of pear, ginger, leek and mandarin, as well as quality control samples generated for an EU-RL proficiency test. UPLC HDMS\(^{[E]}\) drift times generated from the solvent standards and the matrix matched calibrants were shown to statistically belong to the same population. Hence it can be shown that the drift time of the residues is independent of the matrix and can be utilized as a confirmatory parameter to increase confidence in identification. The drift time data generated was entered into a scientific library within a new scientific information system. This allowed the expected and determined drift times to be utilized to reduce false identifications in the proficiency test samples and matrix matched calibrant series analyzed.

Keywords: Food Science, Liquid Chromatography/Mass Spectroscopy, Pesticides, Time of Flight MS
Application Code: Food Science
Methodology Code: Liquid Chromatography/Mass Spectrometry
Food fraud, loosely defined as the deliberate misrepresentation of a product to a consumer for monetary gain, is a growing problem in the global marketplace. One of the most often reported cases of food fraud is olive oil adulteration, including the mislabeling of regular olive oil as extra virgin and the substitution of olive-derived oils with other less expensive edible oils. Detecting these food fraud cases is complicated by the inherent variations in natural products and by the range of potential edible oil adulterants. A method has been developed to characterize edible oils and edible oil mixtures towards the goal of detecting food fraud. Head space solid phase micro-extraction (HS-SPME) was used to sample aroma and flavor profiles of extra virgin olive, olive, peanut, grapeseed, and vegetable oils. The samples were analyzed by LECO’s two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GCxGC-TOFMS). This analytical approach isolated and identified individual analytes within the complex food matrix and provided general characterization of the aroma and flavor analytes. The oil varieties were clearly distinguished through the distinct visual two-dimensional chromatograms and by individual analyte differences. Feature selection determined a set of analytes that differed between the oil varieties to use as variables for Principal Component Analysis (PCA). The oil varieties and oil mixtures were distinguished through PCA, suggesting the potential to detect food fraud with this approach.
Synthetic dyes are widely used in food, cosmetic, pharmaceutical and related industries. Due to the structural variation of the starting material and synthetic process, many commercial dyes are often presented as mixtures of isomers and subsidiary colors. It is, therefore, imperative to efficiently isolate the impurities for subsequent identification and characterization. The challenge in chromatographic resolution of the constituents in a dye mixture mainly arises from their structural similarity. The issue is further compounded by the dynamic range of the impurities with respect to the main compound. In this poster, we present a case study using mass-directed preparative LC to simultaneously isolate multiple impurities from a commercially available synthetic dye. Column chemistry, proper scale-up from the analytical to preparative scale, and the impact of MS detection on overall purification efficiency will be discussed.

**Keywords:** HPLC Columns, Isolation/Purification, Liquid Chromatography/Mass Spectroscopy, Prep Chromatography

**Application Code:** Food Science

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
It has been a long history that garlic is used for medicinal applications, such as treatment of wounds, improvement of heart conditions, and prevention of cancer. Investigations have revealed that the pharmacologic effects of garlic come from allicin, a diallyl thiosulfinate compound found in the fresh garlic aqueous extract. Pharmacologically, allicin is active against many bacteria and viruses, and indicates an outstanding antioxidant activity. In order to understand allicin's pharmacologic effects on cells or viruses in depth, it is very important to quantify the amount of allicin in the fresh garlic extract prior to its medicinal applications. Current analysis methods involve high performance liquid chromatography (HPLC) which often associates with either complicated detection or post-column reaction. A simple and rapid HPLC-UV method is thus developed to quantify the amount of allicin in the garlic extract and to address the thermal and chemical stability of allicin in the garlic aqueous extract. Garlic cloves were first crushed, and extracted by using water. The processed aqueous extracts were then introduced to an Agilent HPLC system with a C18 column for the separation and detection of allicin and other compounds. The results of quantification (obtained from the calibration curve and recovery test of allicin) and the stabilities data of allicin under different environment will be presented. The authors thank California State Polytechnic University Pomona for its support.

Keywords: Analysis, Food Science, HPLC, Quantitative
Application Code: Food Science
Methodology Code: Liquid Chromatography
A study was conducted to determine arsenic species in commercially available prenatal and children’s dietary supplements. An extraction method was optimized based on microwave-enhanced protocol. The optimum conditions were 0.2 g of sample, 5 mL of 0.3 M orthophosphoric acid and microwave heating at 90 °C for 30 min. The extracted arsenic was speciated by ion chromatography–inductively coupled plasma mass spectrometry (IC-ICP-MS). All the dietary supplement samples analyzed in this study (ten different products) were found to contain arsenic in the concentration range 60–531 ng/g. The extraction protocol recovered 63–92% of the total arsenic from these samples. Analysis of the extracts showed that all the samples contained arsenite (As(III)) and dimethylarsonic acid (DMA). Arsenate (As(V)) was found in two of the samples, and an unknown As species was detected in one sample. Spike recovery tests were used to evaluate the effect of the extraction condition on the arsenic species and to validate the analytical procedures. The method was also validated by analyzing a multivitamin standard reference material (NIST SRM 3280). Mass balance assay was made to compare the sum of extracted and unextracted arsenic with the total concentration of the element in the samples.

Keywords: Extraction, ICP-MS, Ion Chromatography, Trace Analysis

Application Code: Food Science

Methodology Code: Mass Spectrometry
Benzo(a)pyrene is a strong carcinogen existing in vegetable oil, scorched food and deep-fried food. This research focused on a typical sample such as vegetable oil and successfully extracted Benzo(a)pyrene in vegetable oil by solid-phase extraction method. The main problem we faced was how to extract hydrophobic Benzo(a)pyrene from oil matrix. Hexane was chosen as the best solvent to dissolve vegetable oil after investigation. And then a couple of material were tried to extract Benzo(a)pyrene from the hexane solution. Finally we applied a material with adsorption based on interaction, and designed it to be Cleanert BAP-3(500mg/6mL). 0.5g vegetable oil was dissolved in 3mL hexane and loaded onto the Cleanert BAP-3 cartridge. 10mL hexane was used to wash the cartridge and 5mL methylene chloride to elute the Benzo(a)pyrene out. After drying the eluant with nitrogen blowing, 1mL acetonitrile was used to reconstitute the sample. The detection was carried out on HPLC with fluorometric detection. Sesame oil is special, it was need to be treated with Cleanert Si(500mg/6mL) firstly. This method was proved by many experiments to be effectively extaction of Benzo(a)pyrene from many kinds of vegetable oil.
Surface selection rule (SSR) is a rule to understand the availability of a band in the spectrum, which is theorized in an electromagnetics manner considering a flat interface. Since IR spectroscopy has a great benefit that a transition moment parallel to the electric field generated at an interface yield an absorption band, SSR is conveniently used to discuss the molecular orientation in a thin film on a flat surface. When the diffuse reflection (DR) spectrometry is employed to monitor molecular adsorbates on a rough surface, on the other hand, a highly complicated spectral pattern appears depending on the angle of incidence and polarization, and no SSR has been available thus far. In the present study, a crossed-Nicol polarization technique is employed to separate the specular reflection and diffuse reflection components. Through the analysis of the angular-dependent DR spectra, SSR of DR spectrometry has readily been obtained, which works powerfully to elucidate apparently complicated spectral patterns reasonably.

Keywords: Surface Analysis, Vibrational Spectroscopy, Characterization, FTIR
Application Code: Materials Science
Methodology Code: Vibrational Spectroscopy
ATR is a widely used sampling technique in FT-IR spectroscopy because little expertise is required on the part of the user. For this reason, forensic laboratories are taking advantage of this sampling technique to collect FT-IR spectra of automotive paints. However, the FT-IR spectrum of a clear coat paint sample obtained by ATR will exhibit distortions, e.g., band broadening and lower relative intensities at higher wavenumbers when compared with its transmission counterpart. This prevents library searching as most library spectra are measured in transmission mode. Furthermore, the angle of incidence for the internal reflection element, the refractive index of the paint sample, and the presence of crystalline water and inorganic contaminants can profoundly influence the quality of the ATR spectrum obtained for automotive paints. A correction algorithm to allow ATR spectra to be searched using IR transmission spectra of the PDQ automotive database will be discussed. The proposed correction algorithm to convert transmission spectra from the PDQ library to ATR spectra is able to address distortion issues such as the relative intensities and broadening of the bands, and introduction of wavelength shifts at lower frequencies, which prevent library searching of ATR spectra using archived IR transmission data.
High Throughput Virtual Slit (HTVS) technology is a revolutionary new design paradigm for dispersive spectrometers that enables slit-like spectral resolution without the associated throughput loss, significantly enhancing performance for any given system size. Quantifying the benefits of HTVS technology can be difficult, especially when determining the enhancement for specific applications. We describe several improvements possible with HTVS technology and show how these benefits apply to specific applications.

Raman chemical identification is a highly sensitive method of determining the chemical makeup of samples, e.g. confirming that a chemical has been correctly manufactured. On a manufacturing line, fast product verification leads to increased quality and quantity—HTVS technology allows for significantly decreased measurement time, providing substantial productivity increases.

Standoff spectral measurement range is generally limited by the collected signal from targets. Slit-based spectrometer designs must balance throughput and resolution requirements, severely impacting system sensitivity when high spectral resolution is required. HTVS technology improves both point source and hyperspectral standoff measurements by mitigating the standard tradeoffs between spectrometer throughput and resolution.

HTVS technology allows for size and weight reductions while maintaining performance. This enables significant enhancements where small and/or lightweight systems are required. HTVS spectrometers have the capability to open entirely new regimes to spectroscopic work.

We will present specific examples illustrating how HTVS technology enhances spectrometer capabilities. Serial dilution experiments show that HTVS-enhanced spectrometers are capable of sensing lower sample concentrations than conventional instruments. Standoff Raman chemical imaging using HTVS differentiates similar-looking pharmaceutical samples on an assembly line proxy.

Keywords: Detection, Imaging, Spectrometer, Spectroscopy
Application Code: General Interest
Methodology Code: Vibrational Spectroscopy
Quantification of individual components in a gaseous stream using FTIR is accomplished using calibration curves of the component. Most readily available calibration curves are of the component in balance nitrogen, due to the fact that some standards cannot be produced in the same balance gas as the sample matrix from a feasibility standpoint or for stability reasons. In reality, however, the balance gas of the measured stream is not always inert. Industrial applications, such as analysis of stack emissions or syngas, may require measurements in non-inert gas matrices, including CO2, methane, or hydrogen.

Calibration curves of several components such as moisture and NOx in a non-N2 balance gas were acquired. Quantification of the components using the new calibration curves was compared to the traditional (N2 balance gas) calibration curve. Results indicate there is an impact on the composition estimated by FTIR analysis. This study therefore provides a quantified source of uncertainty coming from the approach to calibration for FTIR analysis.
Fourier transform infrared spectroscopy (FTIR) is equipped with a Michelson interferometer where light from the polychromatic infrared source is split and combined again and the varying difference in the optical path length results in the interference. Although FTIR is useful, the moving mirror implemented in the Michelson interferometer is susceptible to vibration and makes its equipment less practical to obtain infrared spectra in a mobile manner.

We have developed a novel infrared imaging spectroscopy technology equipped with a near common light path interferometer which consists of no moving parts and can be implemented in as small a spectrophotometer as 20 mm across and 100 mm long. These features can help obtain infrared spectra in a mobile manner with this spectrometer implemented in hand-held instruments.

The parallel light beam collimated by the objective lens of the light from the imaging line in the object plane goes through the phase shifter, partly through the wedge glass and partly through the cuboid glass, before it is focused again by the cylindrical imaging lens. The different light path length between the light beam going through the wedge glass and that going through the cuboid glass results in an interferogram generated along the line in the detector. The infrared spectra can be obtained along the imaging line when the interferogram is Fourier transformed.

In this spectrophotometer, interferograms are recorded only of the lights from the objects located in the focal plane, because the combined light beams interfere only when they are focused. This feature should help ensure highly specific spectra of the light coming from a tiny part of the objects and, when equipped with additional moving capabilities, obtain two or three dimensional spectra.

**Keywords:** FTIR, Infrared and Raman, Portable Instruments

**Application Code:** General Interest

**Methodology Code:** Vibrational Spectroscopy
A Polarization Difference Technique for Surface-Enhanced Infrared Absorption Spectroscopy

Surface-enhanced infrared absorption (SEIRA) is an effect that the IR absorption of molecules adsorbed on metal nanoparticles or rough metal surfaces are significantly enhanced. [1,2] The SEIRA spectroscopy in the ATR mode (ATR-SEIRAS) has been applied most successfully to investigate adsorption and reaction of molecules on metal surfaces. In usual SEIRAS measurements, a spectrum of the bare metal surface recorded before the target molecule being adsorbed is used as the reference. Such measurements are easy in most cases. In studies of the electrochemical interface, for example, a reference spectrum is measured first in the pure solution and then sample spectra are measured after adding the target molecules into the solution. However, such tactics cannot be used in some other cases, for example, in the measurements of self-assembled monolayers (SAMs) on surfaces, because it takes several hours to days to establish SAMs. During the sample preparation, the background spectrum of the metal surface usually changes, which makes it difficult to obtain good spectra.

To remove this problem, we have developed a new method employing the polarization dependence of SEIRA spectra: only p-polarized radiation gives the signal from adsorbed species. [3] Therefore, by taking the ratio of p- and s-polarized spectra, the spectrum of the adsorbed molecules can be obtained without any reference spectra of the bare surface. We will demonstrate the effectiveness of this polarization difference technique.

References

Keywords: Electrode Surfaces, Spectroelectrochemistry, Surface Analysis, Vibrational Spectroscopy

Application Code: General Interest
Methodology Code: Vibrational Spectroscopy
There are at least ten neurodegenerative disorders, including Huntington’s disease, which are caused by genomic CAG repeat expansions that encode for polyglutamine (polyQ) segments in proteins. The aggregation of the expanded polyQ-rich segments within pathological proteins are believed to play a role in cytotoxicity and pathophysiology. Here, the aggregation of the model polyQ peptide system, D[2][Q[10][K[2]] (Q10), is examined using UV Resonance Raman spectroscopy (UVRR). Q10 can be poised to exist in two different solution-state conformations. Lyophilized Q10 peptide powder that is dissolved in water forms [beta] hairpin structures that possess Type I or III turn conformations. Q10 initially treated with Trifluoroacetic acid (TFA) prior to being dissolved in water (the TFA is subsequently removed) exists primarily in polyproline-II-like (PPII) helices and 2.5[1]-helices. Both the structural forms aggregate when incubated at 60[degree]C. The aggregates appear to be fibril-like and exist as different polymorphs. The secondary structures of these two polymorphs are also substantially different. Hydrogen-Deuterium exchange (HX) experiments show that ~40% of peptide bonds in aggregates prepared from the PPII/2.5[1]-helix form of Q10 are inaccessible to solvent, whereas ~70% of peptide bonds are HX-resistant in aggregates prepared from the [beta]-hairpin form of Q10. UVRR data shows that the cores of both Q10 aggregate polymorphs are composed of antiparallel [beta]-sheet structure. Aggregates prepared from [beta]-hairpin solutions exist either as stacks of [beta]-hairpins with Type I or III turns, or as stacks of extended [beta]-strands. In contrast, aggregates prepared from PPII/2.5[1]-helices exist as either [beta]-hairpins with [gamma]-turns or as stacks of [beta]-strands.
This paper reports the very first demonstration of a new ionization process that utilizes 3-nitrobenzonitrile (3-NBN) as matrix. This method, called matrix assisted ionization in vacuum (MAIV) was discovered by Trimpin and co-workers in 2012 (1). The key feature of this new ionization method is that it does not require use of a laser, heat, or a high voltage. The approach simply utilizes exposure of a mixture of the matrix and the sample to the vacuum of a mass spectrometer. Reported here are Fourier transform ion cyclotron resonance results obtained with either a MALDI source or an ESI source with no laser applied in the MALDI source nor any high voltage applied in the ESI source. Under these conditions, singly and multiply charged ions were observed in the mass spectra of samples. Accordingly, there is the possibility of performing electron capture dissociation (ECD) studies under MALDI conditions. A mixture of small peptides including angiotensin 1 & 2, leucine enkephalin, allatostatin, and bombesin was analyzed. Proteins analyzed included bovine insulin, ubiquitin, and bovine serum albumin. Mouse brain tissues were also analyzed. The detailed interpretation of the mouse brain results are not complete at this time. The 9.4 Tesla FTMS used for these studies produced resolving powers between 23,000 and 186,000 for the present set of samples.

High Speed Capillary Electrophoresis Coupled to ESI-MS for the Analysis of Metabolites

Two dimensional gas chromatography coupled to time-of-flight mass spectrometry (2D-GC-MS) is a widespread tool for the analysis of volatile metabolites but the technique is of limited use for the study of non-volatile metabolites. Capillary electrophoresis coupled to high-speed time-of-flight mass spectrometry (high speed CE-MS) provides an alternative approach to the analysis of the metabolome. Capillary electrophoresis is an ideal complement to gas chromatography for the separation of components of the metabolome as it can provide fast, high-resolution separations of many non-volatile analytes inaccessible to a 2D-GC-MS method. Advances in mass spectrometry design allow for the acquisition of tandem spectra at high speed, making modern mass spectrometers suitable for the detection and identification of the very narrow peaks (< 1 second) observed in high-resolution high-speed capillary electrophoresis.

Keywords: Capillary Electrophoresis, Mass Spectrometry, Metabolomics, Metabonomics, Time of Flight MS
Application Code: Genomics, Proteomics and Other 'Oomics
Methodology Code: Mass Spectrometry
There are numerous field measurement problems that would benefit from handheld mass spectrometry including safety and security applications. Miniature cylindrical ion traps (CITs) allow high-pressure mass spectrometry (HPMS) measurements while significantly reducing pumping requirements and enabling mass spectrometry platforms with small size, weight, and power (SWaP). We have demonstrated that mass resolution can be increased at high buffer gas pressures by increasing the RF drive frequency. Decreasing CIT critical dimensions at high RF frequencies reduces the required RF drive amplitude but at the expense of reduced charge capacity. CIT arrays restore charge capacity but dimensional uniformity must be maintained to a certain level to retain mass resolution. We are investigating miniature CIT arrays with radii ranging from 0.167 to 0.500 mm for improved sensitivity and resolution relative to single element CITs.

A differentially pumped vacuum system allowed electron impact ionization and mass analysis at 1 Torr buffer gas pressures, while maintaining electron multiplier pressure below 30 mTorr. Individual CIT array elements with radii of 0.225 mm were analyzed at 1.0 Torr nitrogen buffer gas. Peak widths for p-xylene increased from 3.7 Da with two single CITs to 4.3 Da for the seven-element CIT array. As expected, integrated peak area was a factor of seven higher for the CIT array compared to single traps. With a seven element, 0.167 mm radius CIT array, several VOCs were detected in 1.0 Torr room air while a single CIT was not sufficiently sensitive. Miniature CIT arrays enable HPMS measurements using ambient air as a buffer gas.

This project received support from the Defense Threat Reduction Agency-Joint Science and Technology Office for Chemical and Biological Defense.

Keywords: Instrumentation, Ion Trap, Mass Spectrometry, Portable Instruments
Application Code: Environmental
Methodology Code: Mass Spectrometry
Sirtuins are a class of NAD+-dependent deacetylases with a wide range of protein targets. Among the seven mammalian sirtuins, SIRT1 has been found implicated with many age-related diseases, including cancer, Alzheimer’s diseases, type 2 diabetes, and vascular diseases. SIRT1 modulators are of great interest for their therapeutic potential. Activators expected to promote lifespan were discovered by fluorescent assays. However, subsequent studies proved that the activation effect was entirely fluorophore-dependent. This confusion highlights the significance of label-free high throughput screening (HTS) strategy. Mass spectrometry (MS) is a powerful label-free analyzer. The features of m/z-based identification, high sensitivity, rapid scanning and multiplexing make MS promising for HTS. Its potential high speed, whereas often compromised by slow sample introduction methods has inspired us to seek for alternatives. Droplet-based microfluidics is an attractive sample handling platform. It can reliably and precisely manipulate nanoliter-scale samples. We found that oil-segmented droplets can be directly infused into electrospray ionization (ESI) MS for rapid analysis of discrete samples. The feasibility of our droplet-ESI-MS system was demonstrated by two enzyme inhibitor screenings. In pursuit of higher throughput, we have developed a ‘Mass Spectrometry Plate Reader’ (MSPR). It can generate 3096 droplets from eight microplates at 4 Hz, and sequentially analyze them with a triple-quardupole MS at 2 Hz. The application of MSPR is SIRT1 modulators screening. We chose H3K9(Ac) as the substrate, and optimized assay conditions for direct ESI-MS analysis. An 80-compound epigenetic library was successfully screened. Due to the label-free and in vitro nature of the assay, the hits suggest direct impact on SIRT1 thus interference from indirect effect is avoided. MSPR is now prepared to screen a larger chemical library consisting of thousands of molecules with unknown properties.
To date, small mass spectrometers rely upon engineering conventional ionization sources, mass analyzers, and detectors into a luggable form factor. This approach is shackled to high vacuum requirements and hence turbomolecular pumping technology, which decreases the ruggedness and battery life of the instrument. Our lab has shown that miniature cylindrical ion traps (CIT) operated at elevated buffer gas pressures, > 1 Torr, can be used for small molecule detection, i.e., high pressure mass spectrometry (HPMS). Such pressures are easily achieved by displacement pumps, removing the need for turbo pumps. The reduction in size, weight, and power (SWaP) makes the notion of a handheld mass spectrometer possible.

In addition to ion trap operation at 1 Torr, novel ionization techniques compatible with high-pressure buffer gases such as helium and air are needed. A silicon-based microionizer produced by standard microfabrication techniques has been developed as a small, pressure-tolerant, and low power ionization source. This ion source can be electrically modulated and used for electron impact ionization of analytes both internal and external to the CIT. A custom, differentially pumped mass spectrometer was used to demonstrate ionization and mass analysis at pressures exceeding 1 Torr room air. Additionally, current density measurements as a function of air pressure were also characterized. Power and volume requirements for the microionizer were a factor of 40 and 100 below those of traditional thermionic emitters. Moreover, the microionizer is air compatible while thermionic emitters are restricted to inert buffer gases.

Development of a high pressure, air tolerant microionizer represents a significant improvement in SWaP over thermionic emitters and is another step towards handheld HPMS.

Funding for this project is provided by the Defense Threat Reduction Agency-Joint Science and Technology Office for Chemical and Biological Defense.
Oxidative stress underlies many diseases and processes such as atherosclerosis, diabetes, neurodegenerative diseases and aging. A reliable marker of oxidative stress is protein carbonylation. Previously protein carbonylation was studied by affinity labeling through Schiff base formation coupled with mass spectral analysis. This paper reports a new analytical strategy that is more clinically relevant while circumventing the high cost and lack of sensitivity in current methods. Our goal is to develop a high sensitivity, quantitative tool to determine carbonylation sites in proteins using liquid chromatography coupled with fluorescence detection. Natural oxidation of hemoglobin was mimicked by incubation with a major lipid peroxidation end product (4-hydroxynonenal). The advantage of hemoglobin is that it has a biological half-life sufficiently long to provide a time average of oxidative stress. Reactive carbonyl groups were introduced into proteins \[i\]in vitro[/i] provided a model system that allowed labeling of carbonylated proteins with (1) an amine-bearing dye via reductive amination or (2)hydrazine derivative dyes. Labeled proteins were then tryptic digested and separated on a commercial HPLC system using a C18 column. Trypsin cleavage fragments were examined by MALDI-TOF/TOF while fluorescence intensity provided quantification. Peptide sequence and oxidation sites were determined by MS/MS. In clinical diagnosis, the structure of fluorescent peptides at a certain retention time can be found by referring to previous MS/MS results based on the reproducibility of peptide retention times. This targeting scheme provides an inexpensive and sensitive approach to the use of carbonylation protein biomarkers in clinical diagnostics.

The authors gratefully acknowledge support from the National Cancer Institute (grant number 1U24CA126480-01).

Keywords: Biomedical, Fluorescence, HPLC, Proteomics
Application Code: Biomedical
Methodology Code: Liquid Chromatography
Abstract Text

Cellular studies have observed that upon activation of external stimuli, lipid domains (rafts) laterally assemble on bilayer surfaces, the location where processes like signal transduction and viral infections occur. Supported lipid bilayers (SLBs) have been used as model systems to examine raft formation. While imaging techniques such as atomic force- and fluorescence microscopy have provided insight into stimulus-induced raft formation, they lack the ability to simultaneously monitor and specifically identify critical constituents involved in raft formation without prior labeling.

Mass spectrometry imaging (MSI), a complementary imaging approach, provides information on the identity and spatial locale of molecules on a surface.

Successful studies of lipid rafts with MSI are built on two main pre-requisites: the technique is sensitive enough to detect changes in lipid composition at small imaging footprints and the SLB maintains its fluidic integrity under MSI conditions. Herein reports the first attempt to build SLBs on MS-active porous silicon (pSi) substrates with assessment of the technical limitations within the MSI-SLB method. To assess MSI performance, three pSi-based MS ionization techniques were evaluated: desorption ionization on silicon (DIOS), matrix enhanced-DIOS, and nanostructure initiator mass spectrometry (NIMS). The intra- and inter-substrate reproducibility was determined by monitoring the voltage stability during the pSi etching process and by scanning electron microscopy. Laser desorption/ionization-MS was used to further assess the efficacy, the reproducibility, and the sensitivity threshold for each ionization technique. A concentration gradient containing 1,2-dipalmitoyl-sn-glycero-3-phosphocholine was used to establish the limit of detection (LOD) for each substrate; NIMS exhibited the best sensitivity with a LOD in the low femtomolar region. Fluorescence recovery after photobleaching showed that homogenous, fluid bilayers can be prepared on pSi with diffusion constants in the $10^{-8}$ cm$^2$s$^{-1}$ range. Future work will focus on determining the achievable spatial resolution with the pSi-MSI platform, the stability of SLBs under a high vacuum environment, and the effect of the laser beam on SLB stability. The technical validation of this method will pave the road for future studies of raft domains on SLBs and will help elucidate the spatial organization of molecules within these complex systems.

Keywords: Fluorescence, Imaging, Laser Desorption, Mass Spectrometry

Application Code: Bioanalytical

Methodology Code: Mass Spectrometry
MALDI mass spectrometry imaging (MSI) is a powerful technique to visualize the distribution of a wide range of molecules in biological samples. Although MALDI MSI technique has been rapidly developed in recent years, three challenges remained unsolved: (1) difficulty in protein identification directly from tissue without homogenization and LC MS/MS analysis; (2) difficulty in producing efficient fragmentation from singly charged ions; (3) limited mass range of high resolution and mass accuracy mass spectrometers. Herein, a novel method was developed to overcome these difficulties by combining [i]in situ[/i] enzymatic digestion with multiply charged MALDI MS using a high resolution and mass accuracy mass spectrometer: MALDI-LTQ-Orbitrap. Here, [i]in situ[/i] LysN and tryptic digestions were conducted on thin tissue sections; 2-nitrophloroglucinol, which is a laserspray matrix and can generate multiply charged ions, was applied onto tissue sections using a robotic sprayer system and data dependent analysis was conducted on digested tissue sections. The resulting data was submitted to protein database search for protein identification. LysN peptides are known to yield simplified MSMS spectra in MALDI that are good for [i]de novo[/i] sequencing. By generating multiply charge ions, higher mass molecules were detected and the fragmentation efficiencies were improved. Preliminary results revealed that multiply charged proteins were successfully detected and mapped on rat brain tissues with high mass accuracy MSI. Moreover, abundant sequence-specific fragmentation ions of peptides and protein standards have been observed from multiply charged precursor ions. In conclusion, multiply charged MSI can become a useful and beneficial tool for [i]in situ[/i] protein identification and visualization.
Mass spectrometry analysis of cells and tissue is usually based on bulk samples with the assumption the properties of individual cells are similar; however, this does not account for the heterogeneity of cells and variations that can occur in the distribution of biomolecules. Mass spectrometry is a key analysis technique for cell biochemistry, but there are several technological obstacles that must be overcome for it to be used to its full potential with single cells. Imaging by MALDI and other laser-based methods can approach single cell spatial resolution, but focusing below the micrometer level is challenging. The goal of this research is develop an approach for nanometer scale laser ablation sampling of single cells and tissue coupled with electrospray ionization mass spectrometry. This system uses an atomic force microscope (AFM) stage for apertureless near-field laser ablation and capture in a nanoelectrospray tip. The laser ablation system couples a commercial AFM with a needle tip and pulsed nanosecond laser to ablate samples. The AFM system images the sample and allows the needle to be placed approximately 10 nm from sample surface. The ablated material is collected in a nanoelectrospray capillary tip suspended above the sample. The tip and suspended droplet are viewed using video camera. The ablated material is run off-line on an electrospray mass spectrometer in static nanoelectrospray mode. In the current system volatile molecules have been ablated, captured, and detected by mass spectrometry. The end goal of the project is a system in which individual cells or issue sections are imaged, ablated for MS analysis, and then inspected by AFM after ablation.

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

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
Nano- pipettes have been widely employed for applications that range from small volume delivery to scanning probe microscopies. We have recently demonstrated nanopipette utility as emitters for electrospray ionization mass spectrometry (ESI-MS). Here we describe application of nanopipettes as an all-in-one tool that can accomplish sample collection, contain chemical reactions necessary for analysis, and subsequently serve as the electrospray emitter for analyte detection via mass spectrometry. Samples collected into the tip of a nanopipette are subjected to proteolytic digests and then electrospayed into a mass spectrometer for peptide identification. Use of nanopipettes in this capacity may streamline biological mass spectrometric analysis and show promise in the field of imaging mass spectrometry.

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**Keywords:** Bioanalytical, Electrospray, Mass Spectrometry, Sampling

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
The objective of this research is to develop five isotopic N, N-dimethylated leucine (iDiLeu) mass labels for absolute quantitation of peptides in MALDI and LC-MS analyses. These reagents are structurally identical except for the number of deuterium atoms incorporated into them, and they label peptides by reacting with free N-termini or primary amine side chains. Different concentrations of labeled peptide standards can be combined and then detected in a single MALDI or LC-MS experiment to create a standard curve, increasing the throughput of standard curve generation in MS by five-fold while decreasing quantitative error from run-to-run variability. Reagents were synthesized via reductive dimethylation to incorporate different numbers of deuterium atoms by using various combinations of light and heavy formaldehyde, sodium cyanoborodeuteride and sodium cyanoborohydride. The resulting labels have 0, 4, 7, 10, or 14 deuterium substituents, and molecular structures were confirmed using a Bruker maXis 4G UHR-TOF. Quantitative accuracy of the labels was first demonstrated by labeling a neuropeptide standard containing FMRF-amide, FMRF amide-like peptide I, and Allatostatin at a ratio of 1:1:1:1 and 1:2:3:4:5 and acquiring data on a Bruker ultrafleXtreme MALDI TOF/TOF. Average ratios ranged from 0.94 to 1.03 with a maximum variability of 24%, and constructed calibration curves showed high linearity (lowest $R^2 = 0.989$). On-going experiments will enable absolute quantitation of peptide standards in a more complex biological medium. Chromatographic separation will be performed on a Waters nanoAcquity UPLC while data will be acquired by a high-resolution Thermo Q-Exactive Orbitrap mass spectrometer.

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Chemical tagging of proteins or peptides by stable isotopic reagents is frequently used in MS-based quantification. Recently, our group developed a workflow to combine precursor isotopic labeling with isobaric tagging (cPILOT) to achieve up to 12 (for TMT) or 16 (for iTRAQ) sample multiplexing capability. This workflow was originally demonstrated for the post-translational modification, 3-nitrotyrosine, and has been further extended to global quantitation. Here we present a novel cPILOT workflow based on cysteine tagging in order to simplify complex mixture. After irreversible cysteine tagging, multiple enrichment using antibody-based techniques are employed to generate mixtures of cysteine-containing peptides. Further selectivity is performed using a MS3 HCD method also developed in our laboratory. The details of this workflow and its suitability for enhanced multiplexing of cysteine-enriched peptide mixtures are discussed.
High-throughput \textit{de novo} sequencing is both one of the greatest goals and most daunting challenges in mass spectrometry (MS)-based proteomics and peptidomics. Modern peptide sequencing is dominated by searching MS and tandem MS (MS/MS) fragmentation spectra against databases of genome-predicted protein sequences digested in silico. Although highly successful, the inherent requirement of a sequenced genome and foreknowledge of possible post-translational modifications sustains the desire to sequence peptides directly from MS and MS/MS spectra.

We have recently initiated an in-depth investigation into the potential \textit{de novo} sequencing enhancements provided by our N,N-dimethyl leucine (DiLeu) tags. Although it has previously been demonstrated that dimethylation of the peptide N-terminus increases the yield of N-terminal fragment ions (a-, b-, or c-ions), a dimethylated N-terminal leucine can dramatically alter the backbone fragmentation dynamics. Here, we demonstrate that DiLeu tags can produce simplified MS/MS spectra with minimal secondary fragmentation. With the proper combination of \textsuperscript{2}H, \textsuperscript{13}C, \textsuperscript{15}N, and \textsuperscript{18}O incorporated into a pair of tags, a neutron-encoded sub-Dalton peak doublet will appear for all N-terminal fragments, while fragments containing the C-terminus will appear as a singlet. To further improve the production of a ladder series of b-ions, we have employed a lysine-N protease to cleave proteins into peptides with highly basic N-termini. With the assistance of a customized fragment identification algorithm, we will search the MS/MS spectra of tagged endogenous and digest peptides with PEAKS or PepNovo to determine the precise utility DiLeu has to offer to the field-wide end goal of efficient \textit{de novo} sequencing.
Mass spectrometry imaging (MSI) is a powerful analytical tool allowing multiplex, non-targeted, and label free molecular imaging in biological systems. Secondary ion mass spectrometry (SIMS) enables MSI at submicron spatial resolution for cell-scale investigations, but detecting analytes of interest remains a challenge due partly to low ionization efficiency. This limitation is somewhat alleviated by polyatomic “cluster” projectiles such as C_{60} and Ar_{2000} which enhance molecular ion yield relative to monatomic alternatives, but additional enhancement is possible through optimized sample preparation. Cell stabilization and signal enhancement techniques such as metal-assisted (MetA) and matrix-assisted (ME) SIMS have been explored for "traditional" projectiles such as Au^{+} and Cs^{+}, but they have not yet been thoroughly evaluated for compatibility with newer cluster projectiles.

Here we evaluate and compare the effectiveness of sample preparation and signal enhancement approaches for C_{60}-SIMS imaging of biological samples at high spatial resolution. Experiments were performed on a quadrupole time-of-flight mass spectrometer customized by the addition of a C_{60} ion gun, vacuum chamber adapter, and rectilinear ion guide to enable SIMS-mode operation. Two sample types were used as the basis for comparisons: nervous tissue (rat spinal cord) sections and single neurons from invertebrate [i]Aplysia californica[/i] cultured on silicon wafers. For MetA-SIMS, metal type and thickness were investigated. For ME-SIMS several compounds were evaluated including traditional organic MALDI matrices, and parameters such as coating thickness and deposition method (e.g. sublimation, electrospray) were compared. Preliminary results indicate that several MALDI matrices enhance signal for intact lipid ions, while metal coatings suppress them.

Funding from DoE and ACS analytical graduate fellowship, sponsored by Eli Lilly.

Keywords: Imaging, Mass Spectrometry, Neurochemistry, Surface Analysis
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
A new engineered carbon adsorbent has been developed for use in a range of solid phase extraction (SPE) applications. This adsorbent has unique selectivity due to fullerenes bound to the surface of a porous silica substrate. The resulting stationary phase enables sorption of large molecules such as pigments extracted during QuEChERS while still allowing for excellent recoveries of planar pesticides such as thiabendazole or terbufos. Typically the graphitic carbon black (GCB) used to remove these pigments decreases the recoveries of these planar pesticides and requires additional steps and solvents to resolve this issue. In addition the engineered fullerene carbon adsorbent has decreased retention for large planar molecules which allows for increased SPE performance with larger polycyclic aromatic hydrocarbons and high chlorine count dioxins.
Laser diffraction is the dominant method for characterizing particles since it is fast and can be used for analyzing particle diameters from tens of nanometers to millimeters. For many particle systems the determined particle size is a function of the complex refractive index of the particle. That is, if the correct value of refractive index is used for the calculations, the correct particle size distribution is obtained. However, if an incorrect value of refractive index is chosen, the obtained particle size distribution will be incorrect and that error is a function of the refractive index choice. Therefore, the choice of refractive index is important. Unfortunately, refractive index values are not always readily available and the effect of refractive index errors varies with particle size. For example, the effect of refractive index on determined particle size is widely considered to be small for large particles. Here, the effect of refractive index choice on determined particle size is quantitatively analyzed for a number of realistic particle samples. This data can be used to obtain a rough idea of the importance of refractive index choice on accuracy for various particle size ranges.
Fiber composite materials are widely used in many applications and the fiber/matrix interface is a key area of research. One example is the increased usage of fiber composites in commercial airplanes to reduce weight while maintaining or exceeding the mechanical strength of metallic counterparts. Surface treatments and sizing (i.e. coating of less than 1 µm) can impact the adhesion between the fiber and matrix which can define the mechanical properties of the overall composite. Therefore, understanding the chemical composition at the interface between the carbon fibers and the surrounding epoxy matrix with sub-micrometer spatial resolution is critical for characterizing and enhancing the properties of fiber composites. This requirement itself is another hurdle to surface chemical analysis because the spatial resolution of many techniques is coarser than the scale of the interface. Fourier transform infrared spectroscopy (FT-IR) has been a dominant technique in providing such chemical information, but its spatial resolution is diffraction limited to between 3 to 10 µm depending on wavelength. Atomic force microscope-based infrared spectroscopy (AFM-IR) can improve spatial resolution to ~100 nm and has been previously used to image fibers. In addition by varying the polarization of the incident infrared light the orientation of electrospun PVDF fibers and highly oriented Kevlar® microfibrils has been examined. However, the previous configuration of the AFM-IR instrument used total internal reflection illumination from below the sample and required thin samples (less than 1 µm) to be deposited onto IR transparent optics. In this work, we have employed top side illumination that allowed measurements with substantially simplified sample preparation. The chemical nature at the interface of carbon fiber-epoxy composite is spatially characterized using the modified AFM-IR technique.

Keywords: Infrared and Raman, Materials Characterization, Surface Analysis, Vibrational Spectroscopy
Application Code: Materials Science
Methodology Code: Surface Analysis/Imaging
Hybrid xerogel materials prepared from various compositions of n-octadecyltrimethoxysilane (C18), n-octyltriethoxysilane (C8) and tetraethoxysilane (TEOS) have shown to be useful for control of marine biofouling. Prior characterization by Fourier transform infrared (FTIR) microscopy has shown these coatings result in well-defined segregated alkane-rich and alkane-deficient regions, with what seems to appear as micron scale holes. These results, although significant, are limited to a ~ 3 cm\(^{-1}\) step size and cannot provide sufficient insight into how these materials form or prevent fouling. We report a more precise and efficient analysis of these materials by using co-localized optical, and scanning probe microscopies to reveal structural, electronic, and chemical information at the nanometer length scales. Rigorous analysis includes the application of confocal Raman microscopy to elucidate chemical segregation, complimented by surface potential imaging by scanning Kelvin probe microscopy (SKM) which is collected in parallel with atomic force microscopy (AFM).
**Abstract Text**

Development of sensors for effective detection and discrimination of volatile organic compounds (VOCs) has remained near the forefront of experimental research. In particular, VOC sensors have a multitude of applications in medical diagnosis, environmental monitoring, food safety, homeland security, and other areas. Among different VOC sensing technologies, acoustic wave-based sensors, such as the quartz crystal microbalance with dissipation monitoring (QCM-D), have been widely investigated. We have developed a QCM-D sensor coated with ionic liquid (IL)-polymer composite materials to detect and discriminate a range of VOCs. The frequency change ($f$) and dissipation factor shift ($D$) of the sensor upon exposure to different VOCs were simultaneously measured. Interestingly, we observed a direct relationship between $f/D$, at the first harmonics, and the molecular weight of analyte vapors. To further understand this interesting observation, $f$ and $D$ values obtained for various harmonics were modeled using QTools software provided by the manufacturer, and this has provided insight into the viscoelastic characteristics of these films. In this talk, I will discuss our experimental and theoretical findings, which should be useful for determination of approximate molecular weight of vapors, and should also facilitate an efficient discrimination of vapors based on molecular weights.

**Keywords:** Analysis, Chemical, Sensors, Volatile Organic Compounds

**Application Code:** Materials Science

**Methodology Code:** Sensors
The detection of nucleosides occurs through the development of electrochemiluminescent (ECL) electrospun nanofibers. Electrospinning is a technique that produces nanofibers from polymeric material. An increase in surface area is observed with nanofibers compared to traditional thin films. Creating porous electrospun nanofibers also serves to provide an additional increase in surface area. An ion-exchange polymer, Nafion, has been shown to exhibit high ion exchange capacity. The resulting electrospun nanofibers are composed of Nafion, polyacrylic acid (PAA), and multiwalled carbon nanotubes (MWCNT). Characterization of the electrospun material was performed to determine nanofiber morphology and mat thickness. A chemical possessing chemiluminescent properties, tris(2,2'-bipyridyl) dichlororuthenium(II) Ru(bpy)32+, is then exchanged onto the nanofiber. Nucleoside detection initially proceeds with the oxidation of both analyte and Ru(bpy)32+. As a result, optimal Ru(bpy)32+ loading experiments were conducted and later held constant during analyte detection. The amount of luminescence generated is measured and the degree of luminescence corresponds to the nucleoside concentration. This research was funded by NSEC.

**Keywords:** Biosensors, Luminescence, Materials Science, Polymers & Plastics

**Application Code:** Materials Science

**Methodology Code:** Sensors
It has been previously reported that cationic conjugated polythiophenes offer a label-free DNA sensing platform due to their inherent conformational changes in the presence of nucleic acids. Our group is interested in expanding this unique sensing modality to microRNA (miRNA) in light of its recent potential in disease prognosis. Although a strong fluorescence output is observed from the polythiophene in the presence of double-stranded DNA (dsDNA), there is a technical challenge that arises upon interaction with a miRNA-DNA duplex that results in significant loss of intensity. It is believed this decrease in fluorescence is related to the preferred A-form duplex of miRNA-DNA which alters the polymer conformation in a way that reduces its fluorescence output.

Through preliminary studies, we believe the length, size and chemical functionality of the side-chain groups in polythiophene play important roles in its interaction with various conformations of the double helix. Further, since the primary interaction between the polymers and nucleic acids is believed to be electrostatically driven, environmental stimuli, such as solvent effects and/or the size of the counter ions are also factors that can affect the formation of complexes.

In this presentation we report a systematic study of the conformation changes of the polymer-nucleic acid complex upon modifications to the polythiophene and/or chemical environment. Circular dichroic spectroscopy is the primary tool used in characterization of the conformational differences of various polythiophene derivatives with nucleic acids, while absorption and fluorescence spectroscopy contribute complementary information. In addition, studies into the structural characteristics of the polythiophene derivatives are performed using various characterization techniques, such as dynamic light scattering, static light scattering and size exclusion chromatography in order to provide insight into possible polymer aggregation in the absence and the presence of ssDNA, dsDNA and ssDNA/miRNA. From the data collected, a more thorough understanding of the behavior of cationic polythiophenes is obtained so that structural design of next generation polythiophenes can be tailored for better miRNA analysis.
We present the electrochemical detection of TiO$_2$ nanoparticles (NPs) suspended in methanol. We study bare TiO$_2$ and NPs modified with “N719”, di-tetrabutylammonium cis-bis(isothiocyanato)bis(2,2’-bipyridyl-4,4’-dicarboxylato)ruthenium(II). We have recently reported the detection of individual TiO$_2$ NPs in colloidal suspension (J. Am. Chem. Soc., 2013, 135, p. 10894). We detect the semiconductor NPs using stochastic interactions with a working microelectrode; this was previously reported for electrocatalytic reactions (Xiao X. and Bard, A. J., J. Am. Chem. Soc. 2007, 129, p. 9610). Here, we present the different stochastic interactions that result from the suspended nanoparticles with microelectrodes of different materials: TiO$_2$ and F-doped SnO$_2$. The NPs display distinct electrochemical behavior that range from step-wise current transients to electrochemical oscillations. The effect of NP colloidal behavior on the characteristic electrochemical behavior will be discussed.

Keywords: Electrochemistry, Materials Science, Nanotechnology

Application Code: Nanotechnology

Methodology Code: Electrochemistry
Solid-state stressed degradation kinetics of MLN1117, currently being investigated in a Phase I clinical trials for solid tumors, were quantitatively described as a function of temperature (T) and percent relative humidity (%RH) by using the moisture-corrected Arrhenius equation (Kesselring et al., Waterman et al.) and JMP statistical software. MLN1117 crystalline free base was stored up to 8 weeks at seven stressed conditions. There was no change in physical form detected by XRPD, DSC, or TGA. The main degradation product detected by HPLC analysis at all stressed conditions was identified as a hydrolysis product using high resolution mass spectrometry. The formation kinetics of this hydrolysis product were approximated as zero order and the degradation rate constant (k) was calculated from initial slopes with a correlation coefficient (R²) 0.95. Experimental data were fitted to the moisture-corrected Arrhenius equation (lnk = -Ea/RT + B*(%RH) + lnA, where A, Ea, and B are fitting constants) by using JMP to give the predicted formula: ln k = -11991×(1/T) + 0.0293×(%RH) + 29.893 (Figure 1). The average deviation of the predicted ln k from the experimental value is 4.6%. Both T and %RH were found to be significant effects (p-value in JMP effect test < 0.05) and confidence curves cross the horizontal line of the mean in leverage plots. Four stressed conditions were sufficient to build a model that yields predicted values with reasonable deviation from experiment (e.g. 6.0% for degradation at 37°C/75%RH). This model could be used to predict shelf-life and may aid specification setting.

Keywords: Chromatography, Drugs, Statistical Data Analysis, Thermal Analysis
Application Code: Pharmaceutical
Methodology Code: Computers, Modeling and Simulation
In vitro pharmacokinetic (PK) models offer an appealing, viable option to increase efficiency of the drug development process when working in tandem with animal models. Industry-established diffusion-based models for generating PK curves, such as the hollow fiber chamber reactor (HFCR), successfully mimic PK profiles obtained using animal models. Unfortunately, the HFCR consumes large volumes of drug, thus placing strain on synthetic chemists and recovery of samples is difficult (e.g., the drug-affected cells) for post-exposure analysis. Microfluidic technologies can alleviate some challenges associated with the HFCR, i.e., volume and sample recovery, but the lack of automation potential, present in current pharmaceutical infrastructures, would hinder such devices being used in industry. Fluidic devices created by 3D printing offer a rigid, reusable platform applicable for automated infrastructure present in industry that is based on microwell plates. PK profiles of fluorescein and linezolid (an antibiotic), obtained, using either a fluorescence plate reader or mass spectrometric detection, mimic conventional profiles. Using the diffusion based DIVM, a well (cell culture insert) containing sample was administered a single micromolar dosing of linezolid or fluorescein by diffusion across a membrane. This membrane separates the channel from the well, where initial concentrations of linezolid (5 µM) and fluorescein (10 µM) were flowed at 10 µL/min. Half lives of less than 45 minutes for both compounds were achieved. Importantly, 3D-printed devices, as a whole, offer advantages such as the absence of laboratory steps during creation of the device and electronic file sharing with other laboratories, which has broad reaching implications.

**Keywords:** Fluorescence, Lab-on-a-Chip/Microfluidics, Mass Spectrometry, Pharmaceutical

**Application Code:** Pharmaceutical

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Levothyroxine sodium is an important medication used primarily for treating patients with hypothyroidism. Levothyroxine sodium tablets have been recalled many times since their 1955 introduction to the US market. These recalls resulted from the failure of lots to meet their content uniformity and potency specifications. The purpose of this study is to test the hypothesis that the chemical stability of levothyroxine sodium pentahydrate is compromised upon exposing the dehydrated substance to molecular oxygen. The impact of temperature, oxygen, and humidity storage conditions on the stability of solid-state levothyroxine sodium was examined. After exposure to these storage conditions for selected periods of time, high performance liquid chromatography (HPLC) was used to quantify the formation of impurities. The results showed that levothyroxine sodium samples degraded significantly over a 32 day test period when subjected to dry conditions in the presence of molecular oxygen. However, dehydrated samples remained stable when oxygen was removed from the storage chamber. Furthermore, hydrated samples were stable in the presence of oxygen and in the absence of oxygen. These results reveal conditions that will degrade levothyroxine sodium pentahydrate and elucidate measures that can be taken to stabilize the drug substance.
X-ray photoelectron spectroscopy (XPS) is a qualitative and quantitative surface analysis technique with a nominal sampling depth of ~10 nanometers. XPS can detect all elements, except for hydrogen and helium, and has a detection limit of ~0.05-0.1 atomic percent for most elements. In addition to elemental surface composition, XPS can reveal unique information on oxidation state and chemical bonding that is unavailable from other analytical techniques. XPS can also be combined with argon ion beam sputtering to produce in-depth compositional and chemical state information to depths of several micrometers. Despite its many advantages and unique capabilities as a surface analytical technique, XPS has not been widely used in forensic science for the examination of specimens gathered at the scene of a crime. The main reasons for the lack of forensic studies using XPS are: 1) the lack of standard forensic XPS methods and standard samples for comparison to real world samples; and 2) the historical long analysis times (hours per sample) and large analysis areas (several square millimeters) compared to other techniques such as Raman microscopy and scanning electron microscopy combined with energy dispersive X-ray spectroscopy (SEM/EDS). Advances in XPS instrumentation over the last decade have now improved analysis times to minutes per sample and analysis areas down to the range of tens to hundreds of micrometers. In addition, recently developed argon cluster ion sources now allow "soft" depth profiling of organic and polymeric species with minimal ion beam damage, thus preserving the chemical state information available from XPS. XPS, therefore, has increased potential for new applications in forensic science. In this presentation, XPS and argon cluster ion depth profiling results will be discussed regarding several potential applications for forensic science. Applications to be discussed include surface characterization of gunshot residue (GSR), textile fibers, and hair color residues.

Keywords: Electron Spectroscopy, Forensics, Forensic Chemistry, Surface Analysis
Application Code: Homeland Security/Forensics
Methodology Code: Surface Analysis/Imaging
hiRX is a new technique developed for detection and characterization of plutonium in spent fuel. The spent fuel matrix is a mix of over 50 elements at concentrations below 1%. The major matrix component is uranium from the spent fuel. Hence the dissolved spent fuel solution is a difficult matrix for analytical instruments, considering the radioactivity, high salt content and heavy metal interferences. This combination of analytical obstacles can be overcome using hiRX which uses novel X-ray optics to provide high sensitivity, high selectivity analytical results. hiRX utilizes doubly curved crystal optics to virtually eliminate background and provide a high signal-to-noise response for the analyte element. The implementation of this approach is a prototype instrument using a microfluidic chip as a sample holder. The microfluidic chip holds 1 microliter of sample which reduces the radioactive background significantly and increases the safety margin for the analyst. Analytical results using a synthetic spent fuel matrix to mimic the actual elemental content of a real spent fuel sample along with both a surrogate element and plutonium doped samples will be presented. Analytical precision and accuracy will be studied with the results expected to be well below 5% and 10% respectively. Comparison sample preparation methodologies using the microfluidic chip and the dried spot will also be presented. The authors would like to acknowledge the support of the Next Generation Safeguards Initiative (NGSI), Office of Nonproliferation and International Security (NIS), National Nuclear Security Administration (NNSA).
Defect Induced Mix Experiment (DIME) spherical capsules, utilized as National Ignition Facility (NIF) targets, are composed of a 42 \textmu{}m-thick polymer shell which has been doped with a 2 \textmu{}m-thick inner layer of 1.5 at.% germanium and a 2 \textmu{}m-thick outer layer of 1.5 at.% gallium. The metal-doped layers are separated by a 3 \textmu{}m-thick polymer layer. The LANL DIME campaign requires that the characterization of these capsules must provide better accuracy than the fabrication tolerances.

This presentation will describe the nondestructive characterization of DIME capsules using a variety of X-ray techniques including confocal micro x-ray fluorescence (MXRF) spectroscopy, micro scale and nano scale X-ray computed tomography (CT). While the qualitative characterization of the metal dopant regions of DIME capsules using confocal MXRF is relatively straightforward, the quantitative characterization of these regions is a non-trivial task. In order to quantify the gallium and germanium concentrations, thin gallium and germanium films of sequential thicknesses were fabricated and utilized to calibrate the confocal MXRF instrument. Sample-to-sample variations in metal concentrations were also investigated using statistical analysis of the confocal MXRF data. 3D imaging using micro and nano X-ray CT of DIME capsules provides for improved quantification of the dopant layers by nondestructively measuring the metal-dopant region thicknesses, as well as imaging of induced defects on the capsule equatorial plane.

**Keywords:** Imaging, Materials Characterization, X-ray Fluorescence

**Application Code:** Materials Science

**Methodology Code:** X-ray Techniques
X-Ray Techniques

Analysis for Metals in Nail Polish by Wavelength Dispersive X-ray Fluorescence (WDXRF)

The Food and Drug Administration (FDA) monitors the cosmetic industry under the Federal Food, Drug, and Cosmetic Act. Under this act, companies and individuals who market cosmetic products have legal responsibility to ensure consumers that they are supplying a safe product. The FDA does not require approval of a cosmetic product prior to market release. However, pre-market approval is required for the color additives used in cosmetics.

As the pursuit of beauty focuses on a younger demographic, the importance of minimizing exposures becomes more significant. Although there have been an array of laboratory studies for the volatile chemical content of nail polishes, heavy metals studies have been less frequent.

An assortment of nail polish products from over 20 countries was analyzed for trace metals content. A unique approach was used in the sample preparation of the polishes, which was proven by both WDXRF and ICP-OES analyses. The use of a WDXRF has several advantages in the analysis of these nail polishes, because it has the ability to perform standardless quantitation and is non-destructive, which allows the samples to be characterized by different techniques.

Results for 16 metals along with 14 duplicate preparations shows the WDXRF is a viable analytical tool for the rapid analysis and quantitation of cosmetic samples. Duplicate RPDs were less than 3 percent.

In conclusion, with nail polish being a volatile suspension product this unique sample preparation method controls thickness, homogeneity, and provides excellent reproducibility.

Funding for this research was provided by Research Triangle Institute.

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The Alamo, one of Texas’ greatest historical landmarks, has come to signify Texas’ war for independence. However, the Alamo’s history started roughly a decade before. As a result of recent cleaning of the interior walls, details of wall frescoes and decorations from the first Spanish settlers in the early 1700s have been rediscovered.

Through the use of a portable X-ray fluorescence spectroscopy (pXRF), pigments remaining on the walls were sampled and characteristic elements were identified. Wall markings have been classified into three eras, the Spanish colonial era, the U. S. Army occupation era, and a modern - post 1920 era, based upon characteristic elements found in different pigment samples.

Wall markings in the Sacristy are consistent with pigments used in the Spanish colonial era. A white pigment in the Apse area contains zinc, and is likely from the army occupation area. In the Confessional area, a white coating has been found that contains both zinc and titanium, dating that paint to the modern era. Such analyses can help guide conservation efforts.

In addition to the pXRF, a scanning electron microscope, SEM with EDS capabilities, has also been used on selected sampled fragments. Elemental maps confirm the identification of vermillion (HgS) that is also associated with lead in the Sacristy. This work is supported by the Texas General Land Office, via the Director of the Alamo.

**Keywords:** Art/Archaeology, Elemental Analysis, X-ray Fluorescence

**Application Code:** Art/Archaeology

**Methodology Code:** X-ray Techniques
An instrument is under development for integrating second harmonic generation imaging (SHG) and two-photon excited ultraviolet fluorescence imaging (TPE-UVF) with a synchrotron X-ray diffraction (XRD) beam line, targeting rapid screening for identification and centering of protein crystals. In SHG, two photons of infrared light combine to form one photon of visible light at twice the frequency. The symmetry requirements of this process make it sensitive to non-centrosymmetric crystalline order, including the large majority of protein crystals and excluding most common salts, cryo-protectants, solvents, and aggregated protein. In TPE-UVF, the simultaneous absorption of two photons of visible light, the energy equivalent of one photon of UV light, results in emission of a photon in the near UV to blue range. The wavelengths used are sensitive to aromatic amino acid residues, particularly tryptophan, making ~80% of proteins detectable through a mechanism unique and complementary to SHG. These imaging methods are sensitive to micron-sized crystals, including those grown in the lipidic mesophase that are otherwise difficult to visualize. Positioning by nonlinear optical imaging may reduce or eliminate the need for large area X-ray raster scanning thereby reducing X-ray induced radiation damage and increasing throughput of synchrotron X-ray diffraction. Integration with the synchrotron beam line allows a sample to be mounted on the goniometer, imaged, crystal centered and ready for diffraction in just a few minutes.

**Keywords:** Laser, Microscopy, Protein, X-ray Diffraction

**Application Code:** Bioanalytical

**Methodology Code:** Microscopy
Safety Management in Multidisciplinary Shared Facilities

Research safety management is a major challenge in shared facilities. It is not uncommon to see engineering students working in a shared facility with highly toxic chemical reagents, or biology students working in a machine shop with power equipment. Many of these students have little or no prior experience working in such environments. Worse yet, some of their supervisors do not have the background to provide safety guidance for their students. Many institutions, such as Northwestern University, provide the proper safety training. However there is no efficient way to ensure every shared facility user is properly trained before accessing the multidisciplinary shared facilities.

In this work, we will present the safety measures that we have implemented within the Facility Online Manager® system (http://www.fom.northwestern.edu) at Northwestern University. These measures include, but not limited to,

1. Integration of facility scheduling with the university-wide single-sign-on system. Only authorized users may see the calendar, book time, and access the resources in a facility.
2. Integration of instrument access with the lab entrance door controls. Only authorized users may enter the lab at designated time. All the enter-exit events are recorded in a central database.
3. Integration of facility access with the university safety training records. As soon as the required safety certificate expires, the user’s access is automatically suspended until the certificate is renewed.
4. Remote door controls in the event of an emergency. In case of emergency, University Police may shut the lab entrance doors remotely.
5. More details will be presented at the meeting.

Keywords: Lab Management, Laboratory Automation, LIMS, Software

Application Code: Laboratory Management

Methodology Code: Laboratory Informatics
Determining the optimal reaction conditions and the endpoint of a reaction is crucial to ensure the highest quality yield of the compound of interest and to minimize the formation of secondary products and process impurities. HPLC is recognized as an indispensable quantitative tool supporting Design of Experiments (DoE) to map out the optimal reaction conditions. However, it is also recognized that HPLC is too slow of a technique to provide real-time analysis of reactions. UHPLC provides real-time analytical speed combined with enhanced resolution, sensitivity, and dynamic range but the manual process of extracting and preparing samples significantly delays results. Using a novel process sample manager as part of the UHPLC system, samples are automatically extracted from the reaction vessel and prepared for analysis providing real-time results and superior assay fidelity with improved sample stability, sample solubility and the safe handling of potentially hazardous samples. In this presentation we will discuss the use of UHPLC for quantitative online reaction monitoring of a multi-step 1,4-substituted-1,2,3-triazole synthesis. Continuous flow reactions have many well documented advantages over traditional batch reactions including safety, automation, throughput, and yield. But, direct analysis of continuous flow reactors in real-time has proven to be very challenging. The ability to simultaneously detect and quantitate all components of the reaction (raw materials, intermediates, process impurities, and product) has been elusive. Data will be presented for a DoE optimizing the continuous flow synthesis conditions and monitoring the reaction mixture.

Keywords: HPLC, Lab-on-a-Chip/Microfluidics, On-line, Process Monitoring
Application Code: Process Analytical Chemistry
Methodology Code: Process Analytical Techniques
Ultrapure water (UPW) used in semiconductor factories normally contains 10~40 \( \text{g/L} \) \( \text{H}_2\text{O}_2 \). UPW is usually exposed to ultraviolet light of wavelength around 185 nm to remove organic impurities in the production systems. The radicals (mainly hydroxyl radicals) generated by the UV irradiation cause oxidative decomposition and removal of organic substances from UPW, but some hydroxyl radicals recombine to produce \( \text{H}_2\text{O}_2 \). Because \( \text{H}_2\text{O}_2 \) as impurities in UPW can oxidize materials such as ion exchange resin\(^1\) and metals on Si wafers, it is essential to measure the concentration of \( \text{H}_2\text{O}_2 \) in UPW precisely on the order of \( \text{g/L} \) for semiconductor manufacturing.

Several methods for determining \( \text{H}_2\text{O}_2 \) concentration such as the use of phenolphthalein indicators or peroxidases\(^2\) are in practical use, but these methods require blank water from which \( \text{H}_2\text{O}_2 \) has been removed. To remove \( \text{H}_2\text{O}_2 \) from UPW, we have developed a palladium-loaded monolithic anion exchange resin\(^3\) as a catalyst that is able to remove \( \text{H}_2\text{O}_2 \) in UPW down to below 1 \( \text{g/L} \) at an extremely high flow rate, with no detectable elution of impurities. When \( \text{H}_2\text{O}_2 \)-free UPW made by the catalysts is used as a blank sample and as dilution water for preparing standard solutions, the lower limit of determination of \( \text{H}_2\text{O}_2 \) concentration using phenolphthalein indicator method has reached 1 \( \text{g/L} \).

1) D. M. Prenning, ULTRAPURE WATER, [b]17[/b](3), 49 (2000).

**Keywords:** Calibration, Process Monitoring, Semiconductor, Water

**Application Code:** Process Analytical Chemistry

**Methodology Code:** Process Analytical Techniques
Session Title: General Interests: Lab Informatics, Validation, Software and Process Analytics

Abstract Title: Automatic Twin Vessel Recrystallizer: Absolute Purity Evaluation by Determination of Criterial $T_0$ Value for 100% Pure Compound by DSC

Primary Author: Osamu Nara
Tohoku Pharmaceutical University

Co-Authors

Abstract Text

A novel automatic Pyrex recrystallizer is described. It uses interchangeable twin vessels and eliminates time-consuming recovery and recycling of crystals for repeated recrystallization. The device is very effective in preparing highly pure crystals. The ease and reliability of unattended automatic recrystallization operations and the ability to prepare highly pure crystals make this technique attractive. A novel approach to the determination of absolute purity is also elucidated: this combines automatic recrystallization with differential scanning calorimetry and requires no reference standards. The impurity level of sample tested is computed from van't Hoff’s law of melting point depression:

$$\text{mol}\% \text{ impurity} = 100 \times \left( \frac{H}{RT_0^2} \right) \times (T_0 - T_m)$$

where $T_0$ is the criterial melting temperature of the putatively 100% pure material which can be independently obtained by testing the highly pure product by the repeated automatic recrystallization and $T_m$ is the melting temperature of the sample under test and $H$ is also available from the DSC measurement of the sample.

The purity of six commercial acetaminophen samples and reference standards and an 8x recrystallized product were measured to be 98.8, 97.9, 99.1, 98.3, 98.4 and 99.3 mol%, respectively. The purity of two commercial curcumin samples and a 6x recrystallized product were 87.0, 91.2, and 99.6 mol%, respectively. The present approach offers significant advantages over existing methods of analyzing high-purity chemicals and reference standards.

Keywords: DSC, Method Development, Quality Control, Sample Preparation

Application Code: Quality/QA/QC

Methodology Code: Thermal Analysis
The Bergen Swamp Preservation Society (BSPS) is concerned that the Zurich Bog in Arcadia, NY will be contaminated with waste from a new landfill that is proposed to be built on top of an old landfill near the Bog. There is also concern that contamination has already occurred from the old landfill. To determine if contamination has occurred and to be able to determine if it occurs in the future baseline measurements of the water in Zurich Bog was proposed. Several water samples will be collected throughout the bog using the Environmental Protection Agency (EPA) outlined methods for the collection of waste water samples. Using methods outlined by the EPA the samples will be tested for several selected chemicals deemed as indicators of contamination by the EPA. The methods used for the determination of the concentration of the various chemicals being tested are titration, Flame Atomic Absorption Spectroscopy (FAAS), and Gas Chromatography with Mass Spectrometry detection (GC-MS). The concentration calculated using these methods will be compared to EPA set standards to determine if contamination has occurred from the old landfill. The hypothesis is that the concentration of the chemical selected to be tested will be at low levels because no contamination has occurred yet, to the knowledge of the BSPS. Overall the purpose of this study is to develop a baseline that will be used in the future after the waste facility is built to determine whether contamination has occurred.

Keywords: Environmental/Biological Samples, Environmental/Water, GC-MS, Titration
Application Code: Environmental
Methodology Code: Gas Chromatography/Mass Spectrometry
Formation of 1:1:1 ternary complex species (MAL) has been inferred and the relevant equilibrium has been established using potentiometric technique on the basis of interaction of M2+ ions (M= Cd(II)) with ligand A [A= 2,2’ Bipyridyl amine] in the presence of the ligand L [L = 1-2,1-3 Diamino propane] in aqueous medium at constant ionic strength of 0.2 Mole dm-3 and temperatures 250C. An attempt has been made to for obtain the extent of metal ion distribution among the various species over the entire pH-range of study.
Commercial [styrene-maleic anhydride](SMA)-copolymer was treated with aniline at room temperature for 6 hrs. The resultant Phenylamine pendent [styrene maleic–anhydride] copolymer was designated as Phenylamine styrene malimic acid(PSMA). It was characterized by elemental analysis, -COOH group, -OH group, IR spectral features and thermogravimetry. The polymeric metal chelates of PSMA were prepared with various transition metal ions Cu(II), Ni(II), Co(II), Mn(II), and Zn(II). All the polymeric chelates were characterized by metal: ligand (M/L) ratio, spectral charactivities, magnetic moments and thermogravimetry.

The ion-exchange properties of PSMA were evaluated by batch equilibrium method. For this the effect of electrolytes on metal uptake by polymer, rate of metal uptake by polymer and distribution of metal ion on polymer and mother liquor over wide pH range. The study also was extended to the treatment of metal industries effluents. Ultimately the so called resin can be utilized commercially for the treatment of metal pollutant water i.e. it will save environment.
In this report, we take advantage of a commercially successful technology recently embedded in modern smartphones—Near Field Communication (NFC)—for wireless, non-line-of-sight chemical sensing. To create chemical and physical sensing platforms that have the characteristics of being simple and inexpensive, yet sensitive and quantitative, requires the design of new materials and devices. An enabling approach would be the development of sensing materials and devices that are modular (i.e., readily modified for specific applications), wirelessly addressable, and easily used and interpreted by individuals with no prior technical training. In pursuit of this objective, we have developed a smartphone-enabled sensing platform that utilizes commercially available NFC tags modified with SWCNT-based chemiresistors. The capability of the smartphone sensing system is highlighted by detecting vapors of nitric acid, ammonium hydroxide, and cyclohexanone. Additionally, the ability to monitor cumulative heat exposure is demonstrated by incorporation of a novel thermal dosimeter. Next, the relationship between the readability of the NFC tag and the chemiresistive sensor response is discussed. We conclude by describing outstanding device architecture challenges and opportunities for future chemiresistor development.
With increased number of applications for linear ion trap (LIT) mass spectrometers, the demand for numerical modeling of LIT analyzers has also grown. Such modeling helps to determine functionality trends of an analyzer affected by electrodes design, buffer gas cooling, ion specie, trapping times, and so forth. Knowing these trends can save lots of time when doing optimization experiments with an LIT mass spectrometer.

In this work, we present a novel software simulation package LIT2 for 3D modeling of linear ion traps. It uses boundary element method (BEM) for calculating electrostatic fields, which has been proven to be more accurate for modeling ion traps than finite difference and finite element methods. It contains second order BEM interpolation that provides very high accuracy to be obtained even with a relatively coarse grid. It has user-friendly interface with a separate field solving program that provides high computational efficiency since fields are not repeatedly calculated. LIT2 also supports user-defined dynamic range of voltages, large number of ions (> 400,000) and buffer gas collision effects (hard-sphere and elastic).

The results are given for a non-scanning LIT, targeted for security applications. Due to demands for detecting substances of interest in harsh environments at low concentrations, simulations for optimizing sensitivity were carried. These include optimizations through electrode geometries, buffer gas cooling, trapping times and voltage variations.

Keywords: Ion Trap, Mass Spectrometry
Application Code: General Interest
Methodology Code: Mass Spectrometry
# General Interests: Lab Informatics, Validation, Software and Process Analytics

## Matrix Effects on Boron Containing Materials Due to Laser Ablation Molecular Isotopic Spectrometry (LAMIS)

Laser Induced Breakdown Spectroscopy (LIBS) is a spectroscopic technique that is used for the qualitative and quantitative analysis of materials in the liquid, solid, or gas phase. LIBS can also be used for the detection of isotopic shifts in atomic and diatomic species via Laser-Ablation Molecular Isotopic Spectroscopy (LAMIS). However, the amount of material that is ionized in the plasma depends not only on the applied electric field from the laser but also the physical properties of the material under study. In fact, any additional elements that are entrained into the plasma other than the element of interest, can affect the extent of ablation and quality of spectra and hence, potentially obscure or aid in the relative abundance assessment for a given element. This is common for other laser-ablation optical emission techniques such as laser-ablation inductively-coupled plasma mass-spectrometry (LA-ICP-MS).

To address the importance of matrix effects, the isotopic analysis of boron obtained from boron oxide (BO) emission originating from different boron-containing compounds, such as boron nitride (BN), boric acid (H$_3$BO$_3$), and borax (Na$_2$B$_4$O$_7$·10H$_2$O), via LIBS has been performed here. Each of these materials has different physical properties and elemental composition in order to illustrate possible challenges for the LAMIS method. A calibration-free model similar to that for the original LAMIS work is used to determine properties of the plasma as the matrix is changed.

## Abstract Text
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## Keywords
- Atomic Emission Spectroscopy
- Chemometrics
- Nuclear Analytical Applications
- Plasma Emission (ICP)

## Application Code
- General Interest

## Methodology Code
- Atomic Spectroscopy/Elemental Analysis
Session Title: General Interests: Lab Informatics, Validation, Software and Process Analytics

Abstract Title: Direct Access to Chromatography Data System through Smart Device

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Abstract Text

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, and it is expected that it leads to the solution for various problems and economic growth. Web server to operate CDS from an external terminal is installed on a PC to utilize the new trend with an analytical instrument. The demand to confirm the laboratory status and operate the instrument everywhere and anywhere is realized. Not only monitoring the laboratory status, but also monitoring the real-time chromatogram plot, controlling the instrument, downloading a method and submitting a queue that are required in the daily analysis workflow can be operated from a smartphone and a tablet PC by using this new technology. No special application is required to install into a smartphone and a tablet PC to operate CDS. In addition, in the laboratory where a wireless LAN cannot be used for regulation, it can be operated through the browser of the PC through a wired LAN. This function introduces mobile environment into stand-alone CDS to scale-up to a simple client server system for multiple users. In this study, the improvement of the usability and workflow to reduce the laboratory work by a smart device is demonstrated.

Keywords: Lab Management, Laboratory, Laboratory Automation, Laboratory Informatics

Application Code: Laboratory Management

Methodology Code: Laboratory Informatics
Ceria and zirconia metal oxides have redox properties due to oxygen vacancies which are valuable in several catalytic applications such as conversion of vehicle exhaust [1, 3, 4], production of CO and H[sub]2[/sub] for fuel by solar energy [2], and deoxygenation of pyrolysis oil components for biofuel applications [5]. This work demonstrates how chemical adsorption methods using oxygen, hydrogen, carbon monoxide, propanal, and methanol can be used to characterize the physical and chemical properties of these materials. Oxygen vacancies, or oxygen storage capacity [3], in these materials can be quantified using dynamic oxygen pulse experiments with a thermal conductivity detector (TCD) or a static gas adsorption analyzer. The data show that the oxygen storage capacity can be enhanced when zirconia is incorporated into the ceria, creating a mixed metal oxide. At 300[sup]o[/sup]C, the oxygen storage capacity increased from 40 [micro]mol/g O[sub]2[/sub] to over 70 [micro]mol/g O[sub]2[/sub] when the metal oxide consisted of 25% zirconia. There was no observed oxygen uptake at 200[sup]o[/sup]C. In addition, temperature–programmed reduction profiles of hydrogen showed reduction began above 200[sup]o[/sup]C—consistent with the oxygen experiments—and also provide qualitative structural information about these materials as surface reduction and bulk reduction become indistinguishable with the addition of zirconia. Temperature-programmed-surface reactions of propanal provide information on possible reaction or surface intermediate species, while methanol reactions describe the distribution of acid, base, and redox sites within the materials.


Keywords: Analysis, Biofuels, Chemometrics, Gas
Application Code: General Interest
Methodology Code: Other (Specify)
Analysis of ppb level moisture contamination in Phosphine is necessary to provide the quality of gas required by manufacturers of high performance electronics components. Analysis in a production environment requires a robust method with high sensitivity that is compatible with the matrix gas. In this study, a Fourier-transform Infrared (FT-IR) Spectroscopy method is developed to analyze ppb level moisture in industrial pure grade phosphine cylinders. FT-IR has many advantages over the current method of Process moisture analysis. These advantages will be presented. This is an effective option due to the noninvasive and high sensitive nature of the FT-IR. In addition, the MG2000 work station provides accurate and continuous measurements so that trends can be monitored for a particular cylinder. The majority of the testing performed within this study was conducted on an MKS MultiGas Purity Model 2032 FT-IR Analyzer at a Matheson facility. Initial experiments including linearity, repeatability, reproducibility, and lower detection limit studies of moisture in nitrogen will be presented. The studies concluded with the analysis of moisture doped into the phosphine matrix. The FT-IR proved to be accurate to within ±0.0084 ppm and to have a lower detection limit of 0.050 ppm.

Keywords: FTIR, Water
Application Code: Validation
Methodology Code: Near Infrared
Low quality medicine and fake drug not only cause infant victims in developing countries but produce significant health damage to users who purchase them via net sales. Methods for preventing such counterfeit drugs is crucially required. One approach is fast screening of the pharmaceutical dosage in products. Another resolution is an attachment of special tag which can distinguish genuine products from counterfeit goods. In this presentation, we proposes a system for conveniently creating nano gold beacons that cannot be falsified, duplicated or seen by forgers, and simply detecting such nanobeacons. The nanobeacons comprise of self assembled gold nanoparticles and reporting molecules. Plasmonic properties of well designed noble metal nanostructure performed characteristic emission signals depending on the nanostructure, combination of incident light and molecules, and so on. We have succeeded in a demonstration that about 10 ng gold nanoparticles with $10^6$ molecules showed the identifiable characteristic emission in 1 second irradiation of 785 nm laser. Although conventional absorption and fluorescence spectra cannot avoid broad peak band which leads to limited identification, the peak signals obtained with the nanobeacon were sharp and characteristic since these signals were derived from vibrations of groups in the molecules. Very small amount of gold was invisible to the eyes. Cost of tiny gold is comparable with that of conventional barcode. Robustness including stability and repeatability is still to be investigated. This technology is highly regarded because of the extreme contribution to will make to humankind and society.

**Keywords:** Nanotechnology, Quality, Surface Enhanced Raman, Validation

**Application Code:** Validation

**Methodology Code:** Portable Instruments
General Interests: Lab Informatics, Validation, Software and Process Analytics

Universal Analyzer for Fluidic Systems

During development and maintenance of fluidic systems the knowledge of the current system state is very important. The state is described by the gas flow, the pressure and temperature. This work presents an easy-to-use, flexible, handheld measurement instrument to supervise these values. It contains three flow sensors that measure in a range of -50 to 750 sccm. With its sampling rate of 500 Hz, the stroke of membrane pumps is measurable. Moreover it has three pressure sensors, which measure a differential pressure up to 4100 mbar with a sampling rate up to 2 kHz. Additionally the instrument inherits six thermocouple connectors. They can be calibrated by two semiconductive temperature sensors. Besides the integrated sensors, additional sensors can be connected through four general purpose input and output connectors. The measurement values are presented on a display. Additionally the instrument has an USB, a LAN and a Wireless LAN interface, where the recorded data is sent. For long-term measurements the sampling frequency can be reduced. The instrument can be used battery powered, USB powered or with an external power source. The device and measurements are presented.

Keywords: Gas, Monitoring, Sensors
Application Code: Validation
Methodology Code: Sensors
Modern analytical techniques, like gas chromatography with time-of-flight mass spectrometry (GC-TOF MS), have evolved to the point that complex mixtures can now be analysed routinely. This provides a wealth of information on sample composition; however, it can often be difficult to condense this information into meaningful statistics.

Comparative analysis of complex chromatograms is now an essential part of many routine industrial procedures. For example, quality control of the flavour or fragrance of food-related products or consumer goods may require chromatographic sample profiles to be compared against those of a reference standard or control.

This poster describes a novel software package, designed to simplify the comparison of complex chromatograms, and make it easier to distinguish minor differences. The software converts the total ion current (TIC) or extracted ion current (EIC) chromatogram into a histogram, with components displayed as lines with heights proportional to the deconvolved peak areas. When the chromatogram of a sample is compared to that of a control (reference), a match factor is generated to remove any subjectivity associated with comparative analysis.

This poster reports the successful use of this software to automate chemical profiling of complex mixtures.

Keywords: Gas Chromatography/Mass Spectrometry, GC-MS, Scientific Data Management, Software
Application Code: General Interest
Methodology Code: Computers, Modeling and Simulation
Convolution of Currents at Electroinactive Films on Electrodes

For an electrode modified with inert film where a redox probe partitions from solution into film and undergoes electrolysis at the electrode surface, a convolution method is used to analyze the data. Convolution methods allow extraction of electron transfer kinetics at electrodes in semi infinite solution. This equation is insufficient for film modified electrodes where the diffusion length exceeds the film.

Equation 1 goes here.

Electrolysis of a redox probe A at an electrode modified with our inert film is shown in figure 1.

Figure 1 goes here.

Figure 1. The redox probe A has concentration $C^*$ and diffusion coefficient $D_s$ in solution. In the film, with thickness $l$, A has diffusion coefficient $D_f$ and concentration characterized by the extraction parameter $\Gamma$. On voltammetric perturbation, A undergoes reversible heterogeneous electron transfer at the electrode to become B.

The new technique is not only advantageous for predicting transient currents, but at estimating parameters such as film diffusion length. In addition, convolution methods can discriminate against nonfaradaic capacitance and uncompensated resistance while also allowing rapid determination of reaction kinetics [37]. The model is independent of electrode kinetics and operates under Fick’s laws of diffusion to describe the current-voltage scheme and find the limiting parameters of interest. Throughout the process boundary conditions specific to film solution interface are employed and an explicit finite difference scheme is developed in MATLAB where the cyclic voltammetric data can be imported for the convolution. Overall, the model’s approximation for a particular film thickness will provide improved analysis of voltammograms for inert films or electrodes.

Keywords: Computers, Electrochemistry, Electrodes
Application Code: General Interest
Methodology Code: Computers, Modeling and Simulation
Surface-enhanced Raman scattering (SERS) of probed molecules excited under the waveguide resonance mode, where most of the light is confined in the waveguide layer to generate an enhanced electromagnetic field, is a useful tool for investigating biosensing. In this work, we demonstrate a SERS measurement of protein binding in the porous anodic alumina (PAA) system via waveguide-assisted Raman spectroscopy combing with Ag nanoparticles enhancement (fig.1). The PAA/Al film was fabricated by the partial anodization of an Al film as reported in our previous work.[1] The unoxidized Al (about 20 nm) can improve the coupling efficiency of incident light. Human immunoglobulin (IgG) was first adsorbed to the pore inner walls by covalent immobilization, and a dye (FITC) labeled goat anti-human IgG was bound to IgG through specific recognition. In addition, silver nanoparticles were assembled on the surface of PAA waveguide after analytes assembling, which further enhanced the electromagnetic energy due to surface plasmon resonance of metal nanoparticle.[2]

Fundings: NSFC Grant Nos. 21073073 and 91027010, and National Instrumentation Program (NIP) of the Ministry of Science and Technology of China No. 2011YQ03012408.

References

Keywords: Biosensors, Raman, Spectroscopy, Surface Enhanced Raman
Application Code: Bioanalytical
Methodology Code: Process Analytical Techniques
Many petrochemical HPLC applications are typically performed in mobile phases which are not optimized for Mass spectrometry. In this series of experiments, we will take a traditional HPLC method and use 2DLC to optimize it for MS detection. This gives us an several order of magnitude increase in sensitivity. By using the power of accurate mass, we also can confirm the components and identify unknowns.

The first dimension is the traditional SARA separation, the second dimension moves the automatically any traditionally normal phase Refractive Index responding peaks to the second dimension reverse phase separation with both ESI and APCI detection. In the second phase the mobile phase allows for the addition of a C18 column with an ionizing modifier addition.

**Keywords:** Fuels\Energy\Petrochemical, Liquid Chromatography, Surfactants, Time of Flight MS

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

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Perfluorooctanoic acid (PFOA) is used as a polymerization aid in the production of fluoropolymers. These polymers provide oil and water repellency as well as stain resistance, which make them as ideal coating materials for non-stick cookware. PFOA is bio-accumulative and potentially harmful to humans. PFOA is not supposed to be found in the final products of non-stick cookware after processing. This study presents a method to determine the potential leaching of PFOA from the cookware under simulated cooking conditions. PFOA cookware was extracted with water and ethanol/water mixtures using accelerated solvent extraction (ASE), and the extraction parameters such as pressure, temperature, and time were optimized. The resulting extracts were analyzed by liquid chromatography tandem mass spectrometry (LC/MS/MS).
The production of bioethanol begins with pyrolysis of bagasse, enzymatic conversion into saccharides, and finally fermentation. In order to improve productivity, saccharides as well as inhibitors, such as organic acids and furfurals, of fermentation should be measured.

Typically, saccharides are analyzed by ligand exchange chromatography, while organic acids and furfurals are analyzed separately by ion exclusion/reversed phase chromatography. In order to simplify the analysis, a simultaneous LC/UV/MS method was studied.

A multi-solvent size exclusion chromatography column with polyvinyl alcohol base packing material was used with the optimized eluent condition of 0.1% formic acid/acetonitrile (80/20); the flow rate was 0.6 mL/min, and the column temperature was 50 °C. ESI-MS was used with Selected Ion Monitoring (SIM) mode for detection.

A mixed standard solution was analyzed containing: oligosaccharides (2-5 saccharide units), glucose, glyceric acid, lactic acid, malonic acid, citric acid, pyruvic acid, and maleic acid. The saccharides eluted from the column in accordance with SEC mode, followed by elution of organic acids and furfurals. Retention of the organic acids was attributed to hydrophobic interaction with the packing material rather than SEC mode. Furfurals were not detected by MS, but rather by UV detection.

A sample obtained by pyrolysis of bagasse and enzymatic conversion to saccharides was also analyzed. The analysis showed the standard substances as well as xylooligosaccharides and a mixture of organic acids originated from hemicellulose.

Our method using simultaneous LC/UV/MS is effective for analysis of bioethanol production.

Keywords: Biofuels, HPLC Columns, Liquid Chromatography, UV-VIS Absorbance/Luminescence
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Choline is a water-soluble essential nutrient and is a component of phospholipids that are a major constituent of cell membranes. Choline is a precursor of the neurotransmitter acetylcholine found in living organisms. Their presence is related to several life phenomena and diseases, and it is important to be able to measure the ratio of these molecules.

Typically, reversed phase chromatography is used for analyzing choline and acetylcholine. Due to their hydrophilic nature, an ion-pair regent is utilized for the mobile phase. A post-separation affinity column modified with choline oxidase / acetylcholine esterase, as well as, an electrochemical detector for monitoring hydrogen peroxide are needed to enable highly sensitive analysis. In order to simplify the analysis, a new LC/MS method was studied.

An ion chromatography column, with polyvinyl alcohol base packing material modified with carboxyl groups, was used for the LC. The optimized eluent condition was 4 mM nitric acid / acetonitrile =70/30. The flow rate was 1.0 mL/min, and the column temperature was 30 °C. ESI-MS was used for detection.

The mixed standard solution of choline and acetyl choline (10 ng/mL of each) was analyzed. The retention times of choline and acetylcholine were 5.0 and 6.0 minutes, respectively. Both peak shapes were sharp with baseline separation, and the calibration curve was linear.

Our described LC/MS method should be more facile with higher selectivity than the previous method.
Formation of D-amino acid residues in animal peptides results from a post-translational modification (PTM) in which an L-amino acid is enzymatically converted into a D-amino acid near the N- or C-terminus. Isomerization leads to important changes in the three-dimensional structure and bioactivity of the resulting D-amino acid-containing peptide (DAACP) and increases its resistance to degradation by peptidases. Peptide isomerization is difficult to detect by mass spectrometry and sequencing techniques because no mass change occurs. To address this challenge, we have developed a method for the non-targeted detection of DAACPs in biological samples from Aplysia californica, where two DAACPs have been identified: NdWFamide and GdFFD. Potential DAACPs are identified by resistance to degradation via aminopeptidase M (APM), an enzyme that cleaves one residue at a time and has a higher selectivity for L-amino acids over D-amino acids. More specifically, complex mixtures of endogenous peptides are assayed and peptides that degrade slowly on exposure to APM become candidate DAACPs. Given the difficulty in detecting peptide isomerization, using APM to screen for potential DAACPs offers a rapid alternative to the traditional bioactivity-based approaches. When a resistant peptide is found, we isolate greater quantities of the peptide and acid-hydrolyze it. The chirality of the resulting amino acids is determined using a triple quadrupole mass spectrometer to confirm the presence of specific D-amino acids. Our APM studies indicated 40 peptides from the Aplysia neuropeptidome that resisted degradation; so far, D-amino acids were detected in 7 of these peptides. Because isomerization occurs near the N-terminus of the known molluscan DAACPs, this approach can be applied to other models. To that end, degradation-resistant neuropeptides were identified in the mammalian pituitary, which may indicate the existence of DAACPs in mammals. Funding provided by NIH: P30DA018310 and NS031609.
LC/MS-based proteomics has become a fairly mature technology, and often is applied for practical evaluations in both basic and clinical sciences. An issue is that the obtained data are ensemble averages of many cells so that the average results lose information on cellular heterogeneity. Single cell analyses are becoming more common place with other measurement areas such as genomics, transcriptomics, and targeted proteomics based on signal amplification. These results demonstrate that individual cells have unique characters and should be individually analyzed in some cases. It remains difficult to achieve non-targeted single cell proteome analysis, especially as one cannot comprehensively amplify MS signals of whole digested peptides. Here we optimize each procedure from sample preparation to LC-MS analysis to minimize losses and optimize signals. Using optical tweezers and micromanipulators, target cells are captured into capillary columns packed with immobilized trypsin beads, digested in the capillary column, and then the columns are directly connected to the analytical column. We utilized meter-long and miniaturized capillary columns to achieve high separation efficiency and low flow rate. These conditions reduce ionization suppression effects in electrospray ionization, leading to enhanced MS signals. Overall, we detect hundreds of proteins from minuscule amount of cell lysates, and our ability to perform single cell proteome analysis appears achievable in the near future.

**Keywords:** Liquid Chromatography/Mass Spectroscopy, Proteomics

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

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
In the present study, we evaluated a hepatocyte spheroid array kit as a tool for predicting in vivo drug metabolism by comparing the metabolites of XLR-11, a synthetic cannabinoid, in hepatocyte culture with those in a human urine sample. The commercially available kit contains a specially manufactured multi-well plate and media for three-dimensional hepatocyte culture. This system enables long-term hepatocyte culture that maintains hepatocyte metabolic function. The experimental procedures were as follows: mouse fibroblast cells were used as feeder cells and incubated at 37°C in the plate for 3 days. After the monolayer of feeder cells was formed, HepaRG cells (human liver cell line) were seeded in the plate and incubated for 6 days. Spheroids of the hepatocytes were formed on the feeder cells. XLR-11 was added to the plate at a final concentration of 10⁻⁶ M and incubated for 24 or 48 hours. The medium was collected, and the metabolites were extracted by liquid-liquid extraction after enzymatic hydrolysis. To identify each metabolite, the extracts were analyzed by liquid chromatography/tandem mass spectrometry. A human urine sample obtained from a drug abuser was treated and analyzed in the same way. XLR-11 was metabolized to the monohydroxy metabolite, N-(5-hydroxypentyl) metabolite, and N-pentanoic acid metabolite as well as their hydroxy metabolites by the hepatocyte spheroids. In the human urine sample, these metabolites were also identified, indicating that the in vivo metabolism of XLR-11 was successfully reproduced using the hepatocyte spheroid array kit.

**Keywords:** Bioanalytical, Drugs, Forensic Chemistry, Liquid Chromatography/Mass Spectroscopy

**Application Code:** Bioanalytical

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Data-independent acquisition (MS\[sup\]E[/sup\]) or DIA, a newly introduced mass spectrometric (MS) analysis mode, does not conduct parent ion isolation prior to MS/MS. Rather, the simultaneous MS profiling and MS/MS sequencing is achieved by alternating between low- and high-energy scans. In this study, we evaluated the quantitative performance of MS\[sup\]E[/sup\] by conducting comparative analysis of secretomes of rat vascular smooth muscle cells (VSMCs) in response to pharmacological treatment. Specifically, transforming growth factor \[\beta\] (TGF-\[\beta\]) and its signaling protein, SMAD family member 3 (Smad3) are elevated after vascular injury; up-regulated TGF-\[\beta\] and Smad3 enhance intimal hyperplasia by stimulating VSMC proliferation, migration and extracellular matrix protein production. Rat VSMCs overexpressing Smad3 or green fluorescent protein were treated with or without TGF-\[\beta\]. Secreted proteins were isolated and analyzed by LC-MS. DDA analyses were performed on a Q-Exactive orbitrap and MS\[sup\]E[/sup\] analyses were conducted on a Synapt G2 QTOF MS. MS\[sup\]E[/sup\] identified 296 unique proteins from conditioned media in comparison to 193 unique proteins from DDA in control samples. The average sequence coverage in MS\[sup\]E[/sup\] was much higher than that achieved in DDA mode. About 46% identified proteins in MS\[sup\]E[/sup\] had greater than 30% sequence coverage, whereas the sequence coverage of 50% of identified proteins in DDA was below 15%. Functional analysis revealed that most identified TGF-\[\beta\]/Smad3 induced proteins play important roles in cell proliferation, migration and differentiation. The quantitative results from MS\[sup\]E[/sup\] are complementary to conventional DDA method, enabling discovery of novel biomarkers.
**Abstract Title**
Simultaneous Detection of Eight Urinary Pteridines and Creatinine by Ultra-Fast Liquid Chromatography – Tandem Mass Spectrometry

**Session #** 1670  
**Abstract #** 1670-19  
**Session Title** Liquid Chromatography/Mass Spectrometry Applications

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**Abstract Text**
Urinary pteridines have been preliminarily implicated as potential biomarkers in a number of malignancies including bladder, breast, and lung cancers. Creatinine, which is not affected by diet or by normal physical activities, is commonly used to evaluate glomerular filtration rates and therefore also used as a renal dilution factor in urine analyses. In this study, an ultra-fast liquid chromatography – tandem mass spectrometry (UFLC-MS/MS) method has been developed and optimized for simultaneous detection of creatinine and eight pteridines in urine. This newly developed technique separates and detects eight urinary pteridines, including 6,7-dimethylpterin, 6-biopterin, 6-hydroxymethylpterin, d-neopterin, pterin, isoxanthopterin, xanthopterin, and pterin-6-carboxylic acid as well as creatinine. The method detection limits for the pteridines are between 25 and 300 ng/L (ppt) and 250 ng/L for creatinine. The method was further validated by application to real urine samples from untreated malignant and benign breast cancer patients in a double-blind cancer study. This new method may play a very important role in cancer diagnostics and monitoring. The detailed UFLC-MS/MS method and results will be presented at the conference. This study was supported by Emergence Bioscreening, LLC and Missouri University of Science and Technology.

**Keywords:**  Bioanalytical, Biological Samples, Liquid Chromatography/Mass Spectroscopy, Tandem Mass Spec

**Application Code:**  Bioanalytical

**Methodology Code:**  Liquid Chromatography/Mass Spectrometry
Nitrated and oxygenated polycyclic aromatic hydrocarbons (PAHs), produced through various oxidation pathways from non-polar precursors, are known to be directly mutagenic to human cells. Common approaches to the analysis of these species involve various chromatographic techniques coupled to mass spectrometric (MS) detection. Here we demonstrate and discuss the benefits of LC-APCI-MS with a specific focus on the interpretation of ionization mechanisms (to our knowledge not previously reported) when using high resolution (HR)-MS. In positive mode APCI ionization of nitrated PAHs commonly produce the protonated molecular ion \([M+H]\) along with the common loss of \([m/z] 30\). HR-MS data revealed that this loss was due to either the cleavage of \(-\text{NO}\) or the reduction of the nitro group to its corresponding protonated amine \((-\text{NH}_3^+)\). For the first time it was observed that these two processes were found to be stereoselective among constitutional isomers of 3-ring nitrated PAHs. In negative mode nitrated PAHs were found to have two major ionization pathways (in addition to the associative electron capture by the nitro group): the loss of \(-\text{NO}\) and deprotonation followed by the addition of oxygen. For oxygenated PAHs containing carbonyl groups (i.e., ketone and diketone species), a common loss of \(-\text{CO}\) was observed in positive APCI ionization. It is proposed that this \(-\text{CO}\) loss proceeds through a ring opening mechanism for both 3- and 4-ring oxygenated PAHs. This fragmentation pattern was also, for the first time observed in the APCI analysis of carboxaldehyde derivatives of 3-ring species.

**Keywords:** Environmental/Air, Identification, Liquid Chromatography/Mass Spectroscopy, Time of Flight MS

**Application Code:** Environmental

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
This research assesses the carbohydrate structural differences and glycosylation consistency of a model glycoprotein for application to production lots that are intended for therapies. In the biopharmaceutical industry, there is an increasing interest to understand the effect that carbohydrates have on the functional roles of a glycoprotein. Glycosylation patterns of recombinant glycoproteins are influenced by factors including the expression and growth conditions. These patterns affect the biological activity of the protein, which may subsequently have an effect on the efficacy and safety of the final product.

High performance anion exchange chromatography (HPAEC) coupled with pulsed amperometric detection (PAD) in conjunction with sequential exoglycosidase digestion is used for N-glycan analysis. The system utilizes an anion exchange column and an electrochemical detector. Experiments were carried out to investigate the effect of controlled conditions, such as dissolved oxygen (DO), on an IgG3 antibody produced from cell culture. Three major glycan structures were found and characterized. The monosaccharide profile was determined to contain fucose, glucosamine, galactose, glucose, and mannose. Relative monosaccharide quantities and glycoform heterogeneity will also be studied. The sequence of monosaccharides will be confirmed via fragmentation studies using LC-MS. The system uses a nano-electrospray ionization source coupled to a quadrupole ion trap. Glycoprotein fragments produced by tryptic digestion were separated on a reversed-phase column and the four most intense peaks were isolated for MS/MS fragmentation.

The combined techniques of HPAEC-PAD and LC-MS will be used in conjunction with bioinformatics software to build a library of custom glycan modifications for improved glycoprotein characterization.

*Funding: Meyerhoff Graduate Fellowship Program

**Keywords:** Carbohydrates, Electrochemistry, HPLC, Mass Spectrometry

**Application Code:** Bioanalytical

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Molecular Weight Analysis of Macromolecular Complexes by macroIMS

MacroIMS is a versatile ion mobility spectrometry system that relies on first-principle size analysis to determine the molecular weight of macromolecules and complexes between 8 kDa and > 100 MDa. This technique reduces the breakdown of noncovalent interactions which usually accompanies electrospray ionization through charge neutralization, allowing for analysis of macromolecular complexes. Rapid and reproducible sample analysis produces highly resolved, highly sensitive, and accurate results that are comparable to that of other standard analytical methods. This technique is also independent of the optical properties of the analyte and solvent and can accurately measure multimodal distributions. Here we present the determination of noncovalent interactions between trypsin and its inhibitor (TI) using macroIMS.

The macroIMS system neutralizes droplets produced by an electrosprayed analyte solution, resulting primarily in singly-charged ions. This process reduces the breakdown of noncovalent interactions, allowing for analysis of macromolecular complexes. Ions are sized in a gaseous state via differential mobility analysis in an electrical field and subsequent detection by condensation particle counting. The electrical mobility diameter provided by this technique is highly correlated with the molecular mass of macromolecules over four orders of magnitude. The exceptional resolution was assessed using a commercially available protein mixture at sub-micromolar concentrations. Finally, trypsin and trypsin inhibitor (TI) were analyzed separately and as a mixture (Trypsin+TI) to demonstrate the maintenance of noncovalent interactions.

Keywords: Biological Samples, Chromatography, Electrospray, Liquid Chromatography/Mass Spectroscopy
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Objective. To fingerprint the chemical composition of medicinal Amazonian plants by combining field sampling by solid phase microextraction (SPME) with laboratory analysis by LC-MS. Significance. Although plants from the Amazon have been the source of many great discoveries, there are still numerous species with unknown chemical composition that are used in complementary medicine. Due to environmental problems, many of these species are threatened and might disappear before being investigated. The proposed method allows for a quick and minimally invasive metabolomic investigation of these plants.

Methods. SPME probes were prepared by coating stainless steel wires with a mixture of polyacrylonitrile and RP-amide or HS-F5 silica particles. Sampling was performed by inserting the microextraction probes in various tissues of living plants in their natural environment. After in vivo extraction, the probes were sealed under vacuum and refrigerated until analyzed. The probes were desorbed and analyzed on a Waters Acquity UPLC with triple quadrupole mass spectrometer in positive ion mode.

Results. Twenty Amazonian plant species were sampled and unique metabolomic fingerprints based on principal component analysis were obtained for each of them. In addition, quantitative analysis was performed for previously identified compounds in three species.

Conclusions. In vivo SPME from live plants combined with LC-MS analysis can provide a powerful method for quantitative analysis, identification, and metabolomic fingerprinting of plants. This approach causes minimal damage to the plants, prevents sample degradation, and is much faster than traditional sampling methods.

Funding: Albany College of Pharmacy and Health Sciences

Keywords: Biological Samples, Liquid Chromatography/Mass Spectroscopy, Natural Products, SPME
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Liquid Chromatography/Mass Spectrometry
Active Flow Technology (AFT) is a new format of chromatography column that yields significantly improved chromatographic performance. In AFT the flow of the mobile phase is dynamically managed as it passes through the column, to eliminate wall effects and minimise solute band broadening, therefore maximising signal response and theoretical plates [1]. In parallel segmented flow (PSF) the mobile phase is managed at the column outlet only, in curtain flow (CF) it is managed both at the column inlet and outlet to create a “virtual” column [2,3] inside the analytical column. The “virtual” column has a narrower diameter, the dimensions of which are related to the volumetric ratio of flow exiting the column through the centre, relative to the flow exiting through the peripheral zones.

The practical advantages of using curtain flow have been described in the literature for fully porous [2,3] and monolithic materials [4]. In the work presented in this paper we report a study comparing the efficiency and sensitivity of several column configurations (standard, PSF and CF) where the columns are packed with solid core particles.

References:

Keywords: Chromatography, High Throughput Chemical Analysis, HPLC Columns, Liquid Chromatography/Mass S
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Protein 3-nitrotyrosine (P-3NY) represents a low abundance post-translational modification of protein tyrosine (P-Y). This modification can result in an alteration of protein structure and function and has been associated with various age related pathologies such as atherosclerosis, neuropathies and others conditions. While it is unknown weather P-3NY fundamentally is responsible for disease related pathologies, at present it is considered as an emerging cardiovascular risk factor.

Over the past decade, several approaches have been described for the determination of this bioanalytical target. The present approach relies on the reduction of the 3-NY residue to the corresponding 3-amino-moiety (3-AY), with subsequent chemical modification oriented towards the attachment of various substituents for the purpose of enhanced isolation and/or detection by various techniques. Subsequent to reduction 3AY is elaborated to a 2-phenyl-6-amino (or substituted amino)-benzoxazole (PBO) product. This transformation can be accomplished with benzylamine (BA) or 1,2-diphenyl-1,2-diaminoethane (DPE) with a major product being formed in each case. Present work is focused on the development of a more water soluble substituted DPE, wherein one may realize improved MS properties, maximize single production formation, and allow for the incorporation of stable isotopes.

From the LC perspective, additional activities include the implementation of an Extreme Ultra-Pressure LC (XUPLC) system (typical operation, 30,000 psi; column dimensions, 75 µm x 0.5-1.0 m; packed with 1.9 µm BEH C18 particles). The overall goal is to combine the advances in pre-column derivatization with a chromatographic system providing significantly enhanced peak capacity for investigations of post-translational modifications leading to the formation of P-3NY in samples of biological origin.
The use of liquid chromatography-high resolution mass spectrometry-based screening methods enable the screening of hundreds of contaminants in a single run. There are different parameters affecting the performance of LC-HRMS screening methods including chromatographic, data acquisition and data processing parameters. Mass spectrometric acquisition and chromatographic parameters of the screening methods should be optimized and have an import impact on the method performance, including both sensitivity and selectivity (limits of detection, matrix effects, etc.). In addition, data processing parameters such as automated searching parameters (eg. m/z error tolerances, retention time windows, minimum intensity values, ...) using specific LC-HRMS software are also important to avoid the change of false negatives/false positives. The aim of this work is to evaluate different chromatographic, data acquisition and data processing parameters that affect to screening methods. This study has been performed using an LC-TOFMS instrument. The database comprises retention time and accurate-mass values for 600 compounds. An UHPLC system was used with a C18 analytical column of 50 mm x 2.1 mm and 1.8 um particle size. Different retention time windows and m/z tolerances of the automatic search tool were tested. In addition, 20 representative compounds were selected to optimize chromatographic and acquisition parameters. The chromatography was evaluated using different columns with different sizes, operating at different pressures. Then, various elution gradients were assayed. Different injected sample volumes were evaluated to study the influence on both matrix effects and sensitivity. Mass spectrum acquisition rate was also considered (from 0.5 to 10 Hz). Finally, several compounds with similar retention times were selected to evaluate potential coelution problems in this type of large-scale screening methods.

Keywords: Food Science, Liquid Chromatography/Mass Spectroscopy, Pesticides, Time of Flight MS
Application Code: Food Science
Methodology Code: Liquid Chromatography/Mass Spectrometry
Fast online comprehensive two-dimensional liquid chromatography (denoted LC×LC) is currently attracting a lot of interest due to its markedly higher resolving power as compared to a one dimensional separation. Resolving power is measured in terms of the peak capacity, defined as the number of well-resolved peaks that can be fit in a chromatogram. In an ideal LC×LC separation, the total peak capacity of the separation is the product of the first dimension peak capacity and the second dimension peak capacity. To achieve this ideal peak capacity two conditions must be met: 1) the first dimension sampling and subsequent second dimension chromatogram must be done fast enough to minimize and preferably totally avoid under-sampling the first dimension and, 2) the sample retentions must be spread over the entire two dimensional (2D) separation space, In our online approach to LC×LC, the first goal is achieved by operating the second dimension column at a high temperature (100oC) and very fast flow rate (3 mL/min). Under these aforementioned conditions, we have been able to reduce the cycle times dramatically (e.g. to 12 to 21 seconds) and achieve 2D run times of approximately 30 minutes per sample. Therefore, these conditions for the second dimension have enabled us to perform fast online LC×LC. Unfortunately, these conditions are not compatible with mass spectrometry and pose several challenges.

A major challenge is that a flow rate of 3 mL/min is generally excessive for the ion source of the mass spectrometer to remove enough solvent to allow for adequate ionization of the sample molecules. We are investigating the use of an active flow splitter versus using a column with smaller internal diameter (but the same linear velocity) in order to control the flow rate into the MS. The two approaches will be compared based on the ability to generate peak capacity in each mode.

Keywords: Chromatography, High Temperature, HPLC Columns, HPLC Detection
Application Code: General Interest
Methodology Code: Liquid Chromatography/Mass Spectrometry
In recent years ethyl glucuronide (ETG) and ethyl sulfate (ETS) have gained popularity as biomarkers for alcohol consumption and as a means to document alcohol abstinence. These compounds are direct metabolites of ethanol and can be detected in urine for 3-5 days after exposure, long after blood alcohol concentrations have been eliminated from the body. The detection of these metabolites has proven advantageous for zero tolerance treatment programs and abstinence enforcement where information regarding recent alcohol consumption is required. A simple, fast, rugged, and sensitive method has been developed for the detection of ETG and ETS in human urine by LC-MS/MS in reversed phase mode.

As an alternative to centrifugation or SPE cleanup, urine samples are prepared using a ‘dilute and shoot’ method by directly adding sample into a Thomson Single-Step PVDF 0.22μm filter vial containing mobile phase and deuterated ETG and ETS as internal standards. The samples were analyzed on a Shimadzu UFLC-XR HPLC equipped with an ABSCIEX 4000 LC-MS/MS using electrospray ionization in negative ion mode. The compounds are separated from major matrix interferences using water and methanol mobile phases modified with 0.1% formic acid and 2mM ammonium formate under gradient conditions on a Restek Biphenyl column.

Keywords: Biological Samples, Clinical Chemistry, Forensics, Liquid Chromatography/Mass Spectroscopy
Application Code: Clinical/Toxicology
Methodology Code: Liquid Chromatography/Mass Spectrometry
Caffeic acid is a naturally occurring carboxylic acid present in a variety of plants, including tobacco, and is listed as one of the 93 harmful and potentially harmful constituents in tobacco product and tobacco smoke. Due to being semi-volatile, this compound may transfer to mainstream smoke during cigarette smoking. For this study, 50 brands of cigarettes commonly marketed in the U.S. were analyzed for the presence of caffeic acid in mainstream smoke. Results showed that the concentration of caffeic acid in these samples were lower than the limit of detection of the method (10µg/g). These results support the hypothesis that caffeic acid is decomposed at high temperatures during the combustion of tobacco in cigarettes. The same analytical method was applied to the quantification of caffeic acid in the tobacco filler of cigarettes. The method included a quantitative analysis using high-performance liquid chromatography and tandem mass spectrometry.
Mixed mode interactions have been used for more than a decade in the detection and quantification of smaller molecules in complicated matrices such as human biological fluids. Mixed mode stationary phase columns have been introduced recently in combination with the core-shells particles columns offering a new tool for advanced selectivity and speed analysis that is compatible with LC-MS technology. Modern mixed-mode columns offer great flexibility, reproducibility and loadability for the separation of a wide range of compounds but till today we could not find an application of these columns for the separation of isomeric small molecules.

Therefore in this method we are investigating the application of a mixed mode chromatographic column (Acclaim Surfactant Plus by Dionex, Thermo) for the separation of 12 mono-hydroxylated brominated biphenyls (OH-BDEs) in human plasma in contrast with 12 other chromatographic columns in single or tandem mode. The mix mode column, through different hydrophobic and/or ionic properties (cation or anion) provided additional selectivity of separation. For our chromatographic separation a lower pH (pH=2) was applied in a buffered mobile phase in order to keep the hydroxylated molecules not ionized, that would increase their separation through the reverse phase and cation-exchange mechanism (enhancement of ionic interaction). The chromatographic run was completed in only 15 min with 2 minutes equilibration time. The limits of detection for most congeners were <8 ng/mL except for 4-OH-BDE90 and 6'-OH-BDE99 that the LOD is 60 pg/mL. Each column was used providing accurate results for up to 800 samples.

Disclaimer: The views expressed herein are those of the authors and do not necessarily reflect those of the Department of Toxic Substances Control, California Environmental Protection Agency.

Keywords: Bioanalytical, Chromatography, HPLC Columns, Ion Chromatography
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography/Mass Spectrometry
In the last decade plasma phospholipids and proteins have been associated with matrix effects in liquid chromatographic methods of bioanalysis. When a complicated biological matrix such as blood is injected onto LC-MS/MS, proteins and phospholipids have been shown to reduce the column lifetime and sensitivity, while affecting a method's accuracy.

Protein precipitation protocols (PPT) (salt induced precipitation, addition of organic solvent and others) used initially for the recovery of proteins have been traditionally used for sample clean up for the extraction and recovery of smaller molecules since they are rapid and do not require method development. The technique of liquid liquid extraction (LLE) using a number of organic solvents has been successfully implemented for the removal of phospholipids, but has not been associated with the good recovery for several of the compounds.

In this study we are presenting a novel strategy to determine Bisphenol A, Tetrabisphenol A and other brominated phenols with LC-ESI-MS/MS in human plasma using a Phree Phospholipid removal plate minimizing matrix effects (phospholipids and proteins) in the bioanalytical assay. A systematic comparison of several protein precipitation and lipid crash protocols in 96 well plates was conducted in contrast to a conventional solid phase extraction protocol. In order to ensure fair comparison several different commercial serum samples spiked with the compounds of analysis were prepared with all techniques. Recoveries of all analytes ranged from 85-110%.

Disclaimer: The views expressed herein are those of the authors and do not necessarily reflect those of the Department of Toxic Substances Control, California Environmental Protection Agency.

Keywords: Bioanalytical, Environmental/Biological Samples, Optimization, Sample Preparation

Application Code: High-Throughput Chemical Analysis

Methodology Code: Liquid Chromatography/Mass Spectrometry

Proton-Transfer-Reaction Mass Spectrometry (PTR-MS) is one of the most sensitive methods for real-time detection of VOCs. One limitation, however, is the lack of unambiguous compound identification since only the m/z of an ion is determined. We recently tested several approaches of increasing the selectivity via varying the reduced electric field strength (E/N) and switching the reagent ions between H⁺, NO⁺, O₂⁺, and Kr⁺, respectively. The latter is only possible with the latest generation of PTR-MS instruments, which have been called Selective-Reagent-Ionization (SRI-MS) instruments in literature. We present data obtained with such an SRI-MS instrument on two isomeric new psychoactive substances (4-methylethcathinone and N-ethylbuphedrone). Proton transfer from hydronium to these isomers results in the formation of protonated parent ions with exactly the same mass, i.e. 4-MEC and NEB are not distinguishable. By switching to NO⁺ chemistry we find that the most abundant product ions are fragments at m/z 72.08 and 86.10, respectively. This means that we can clearly separate the isomeric drugs with an SRI-MS instrument.

In order to improve the selectivity even further, we coupled a micro-capillary column (MCC) to a PTR-MS instrument. The MCC, which is small enough to be installed inside a standard instrument and allows for fast spectral runs for near real-time analysis, enables virtually unambiguous identification of isobaric and isomeric compounds, which is demonstrated with several examples (e.g. p- and o-xylene).

In addition to these serious advancements in selectivity, we also present the latest data on the sensitivity and the Limits-of-Detection (LoD) achievable with such an SRI-MS instrument. By increasing the pressure and the voltages applied to the drift tube, the sensitivity for e.g. trichlorobenzene exceeds 500 cps/ppbv; this results in a LoD (3σ) of about 200 ppqv (figure below).

Keywords: Chemical Ionization MS, Drugs, Environmental/Air, Ultratrace Analysis
Application Code: General Interest
Methodology Code: Mass Spectrometry
This paper presents a novel splitting method for liquid chromatography/mass spectrometry (LC/MS) application, which allows fast MS detection of LC-separated analytes and subsequent online analyte collection. In this approach, a PEEK capillary tube with a micro-orifice drilled on the tube side wall is used to connect with LC column. A small portion of LC eluent emerging from the orifice can be directly ionized by desorption electrospray ionization (DESI) with negligible time delay (6-10 ms) while the remaining analytes exiting the tube outlet can be collected. The DESI-MS analysis of eluted compounds shows narrow peaks and high sensitivity, due to the extremely small dead volume of the orifice used for LC eluent splitting (as low as 4 nL) and the freedom to choose favorable DESI spray solvent. In addition, online derivatization using reactive DESI is possible for supercharging proteins and for enhancing their signals without introducing extra dead volume. Unlike UV detector used in traditional preparative LC experiments, this method is applicable to compounds without chromophores (e.g., saccharides) because of using MS detector. Furthermore, this splitting method well suits monolithic column-based ultra-fast LC separation at a high elution flow rate of 4 mL/min.
Determinition of Internal Energy Distributions for Laser Electrospray Mass Spectrometry Using Thermometer Ions and Other Biomolecules

The internal energy deposition for femtosecond laser vaporized dried and liquid samples using laser electrospray mass spectrometry (LEMS) were determined and compared with conventional electrospray ionization mass spectrometry (ESI-MS). LEMS combines 800 nm, 1 mJ femtosecond laser pulses for nonresonant, multiphoton vaporization of samples from a surface with an electrospray source for analyte capture, postionization, and mass spectrometry. The internal energies of the mass spectral techniques were calculated by plotting the survival yield (SY) as a function of collisional energy using benzylpyridinium ions and peptides. Measurements of dried p-substituted benzylpyridinium ions using 1 mJ laser pulses resulted in prevalent fragments other than the respective benzyl cation that led to a greater extent of fragmentation. The mean relative internal energies, $<E_{int}>$, were determined to be 1.62, 2.02, and 1.58 eV and for ESI-MS, dried LEMS, and liquid LEMS studies, respectively. The 24.5% increase in $<E_{int}>$ for dried LEMS compared with conventional ESI-MS most likely occurred due to two-photon resonances owing to the benzylpyridinium ions’ absorption features at 400 nm. The large $<E_{int}>$ difference between dried and liquid LEMS experiments suggest different vaporization mechanisms. Subsequent studies using femtosecond laser pulses with pulse energies < 100 nJ resulted in mean internal energies better than, if not equal to those obtained with nanospray experiments. In studies with larger biomolecules using 1 mJ pulses, LEMS analyses of dried samples showed a decrease in survival yield compared with conventional ESI-MS for leucine enkephalin, and bradykinin of ~15% and 10%, respectively. Survival yields did not decrease with increasing collision energy in liquid LEMS analyses, resulting in improved survival yields in comparison with ESI-MS for benzylpyridinium ions and large biomolecules.
Rapid and accurate identification of bacterial and yeast isolates is needed to advance public health. Touch spray is a novel ambient ionization method developed. It is performed by using a probe capable of transferring material from the object of interest to the surface of the probe for analysis. In this particular application, the bacterial colonies are directly sampled from culture dishes. Touch spray ionization can also be used to detect suspended micro-organisms in various solvents, such as methanol, water, dimethyl formamide and acetonitrile. Detected lipid profiles are characteristic for various bacteria genera. Touch spray has been utilized efficiently to distinguish among gram-negative and gram-positive bacteria in both of positive mode and negative modes. As an example of species differentiation, Staphylococcus aureus, Staphylococcus hominis, and Staphylococcus epidermidis are readily distinguished through their lipid patterns utilizing multivariant statistical analyses. In a parallel study, individual bacterial colonies are transferred to a filter paper cut into a sharp point and analyzed using paper spray-MS. Comparison of the mass-spectral data obtained from paper spray and touch spray ionization techniques show high degree of similarity and reproducibility.

**Keywords:** Biomedical, Food Science, Identification, Lipids

**Application Code:** Food Science

**Methodology Code:** Mass Spectrometry
Development of portable mass spectrometers for homeland security, defense, and environmental monitoring has generated considerable interest. Our laboratory has made significant progress in developing hand portable mass spectrometers utilizing cylindrical ion traps (CITs) operated at significantly higher pressures (~1 Torr) than conventional systems (<1 mTorr). Operation at such pressures allows elimination of turbomolecular pumps, providing a significant reduction in system size, weight and power (SWaP) and improving system robustness. Prototypical handheld systems have been assembled, weighing less than 2 kg, including batteries for 5-6 hr operation.

The use of air as the buffer gas is logistically advantageous in a hand-portable instrument. Additional knowledge gained from tandem mass spectrometry may be crucial to compound identification. Nitrogen and oxygen neutrals, the primary species in air, provide a different type of collisional interaction than helium due to the larger size and polarizability of these molecules.

Analytes ranging from small organic molecules to peptides have been analyzed and fragmented via collision-induced dissociation (CID) in a custom instrument operating with unprecedented pressures up to 1 Torr of air. Electron impact ionization is performed in a microscale CIT with a 0.500-mm radius at a drive rf near 7 MHz. The trap is operated in a chamber held at unprecedented pressures of up to 1 Torr by leaking ambient air into a differentially pumped chamber while an electron multiplier is used as a detector in a chamber held below 10 mTorr. Mass analysis is performed via mass selective instability scanning. Early results show similar fragments from high pressure and low pressure CID.

Keywords: Ion Trap, Mass Spectrometry, Portable Instruments, Tandem Mass Spec
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
Ion mobility spectrometry (IMS) is widely used in national defense to detect explosives and chemical warfare agents. However, field deployed instruments are subject to various field conditions such as varying humidity of the sample and drift gas. Varying water content of the drift gas can significantly alter the drift time and subsequently the mobility constant ($[i]K[0]$) of the ion, influencing the accuracy and false alarm rates of the instrument. While the effect of drift gas water content has been previously investigated, no means to quantify it under field conditions have been developed. In this work, using an IMS-tof-MS instrument capable of high precision measurements of $[i]K[0]$ values within ±0.005 cm$^2$V$^{-1}$s$^{-1}$ or better, we investigated the protonated monomer and proton bound dimer ions of dimethyl methylphosphonate (DMMP) as standards that are sensitive and insensitive to water content, respectively. It was found that the ratio of mobilities of these two ions is a practical measure of the water content of the drift gas and may be used to reduce false alarm rates. The effects of water vapor when ammonia is used as a dopant have been assessed, and interactions of the DMMP monomer with successive number of waters were also modeled. This study was conducted at atmospheric pressure and at temperatures from 30°C to 150°C. Funding was provided by Science Applications International Corporation as a subcontract under a Task Order contract with U.S. Army Edgewood Chemical Biological Center and has been continued with the U.S. Army Research Laboratory’s Army Research Office.

Abstract Text
Ion mobility spectrometry (IMS) is widely used in national defense to detect explosives and chemical warfare agents. However, field deployed instruments are subject to various field conditions such as varying humidity of the sample and drift gas. Varying water content of the drift gas can significantly alter the drift time and subsequently the mobility constant ($[i]K[0]$) of the ion, influencing the accuracy and false alarm rates of the instrument. While the effect of drift gas water content has been previously investigated, no means to quantify it under field conditions have been developed. In this work, using an IMS-tof-MS instrument capable of high precision measurements of $[i]K[0]$ values within ±0.005 cm$^2$V$^{-1}$s$^{-1}$ or better, we investigated the protonated monomer and proton bound dimer ions of dimethyl methylphosphonate (DMMP) as standards that are sensitive and insensitive to water content, respectively. It was found that the ratio of mobilities of these two ions is a practical measure of the water content of the drift gas and may be used to reduce false alarm rates. The effects of water vapor when ammonia is used as a dopant have been assessed, and interactions of the DMMP monomer with successive number of waters were also modeled. This study was conducted at atmospheric pressure and at temperatures from 30°C to 150°C. Funding was provided by Science Applications International Corporation as a subcontract under a Task Order contract with U.S. Army Edgewood Chemical Biological Center and has been continued with the U.S. Army Research Laboratory’s Army Research Office.

Keywords: Mass Spectrometry, Portable Instruments, Quantitative, Water
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
Liquid Sample Desorption Electrospray Ionization Mass Spectrometry (DESI MS) of Analytes in Aqueous Solutions

Liquid sample (LS) DESI relies on electrospray (ES) for sample ionization and allows the separation of sample solution (liquid sample) from the solvent that generates ES. In this work we tested the ionization efficiency of LS DESI of the liquid samples of analytes dissolved in aqueous solutions containing an electrolyte. The liquid sample was placed in a capillary positioned perpendicular to the electrosonic spray (ESSI), ca. 3 mm away from the MS inlet. Using 50/49/1 vol% MeOH/H2O/HAc, pH 4.3 as the ESI carrier solution, the parent ions of dopamine (DA) (1 mM to 2.5 mM) ([DA+H]+, 100 M tyrosine ([Tyr+H]+) and 100 M guanine ([Gua+H]+) was detected in 99/1 vol% H2O/HAc pH = 2.7, [HAc] = 0.174 M. 100 M DA was also detected in 31 mM phosphate buffer (PBS) pH = 7.4. For 100 uM Tyr the relative sensitivity of ESSI, LS DESI, and ESI was approximately the same while the sensitivity of DA was higher in ESI than LS DESI. The presence of electrolyte in the aqueous sample solution had no detectable effect on the sensitivity of DA ionization form aqueous solutions. In addition, in CONC of GUA Gua proton bound dimer ([2Gua+H]+) parent was detected. The results indicate the feasibility and potential limitations of liquid sample DESI.

Keywords: Electrochemistry, Electrospray, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
In this work, a technique has been proposed to achieve simultaneous peptides/proteins enrichment and washfree self-desalting on a sample support with a circle hydrophobic/hydrophilic/hydrophobic pattern. Upon deposition, the sample solution is first concentrated in a small area by repulsion of the hydrophobic outer layer, and then, the peptides/proteins and coexisting salt contaminants are selectively captured in different regions of the pattern through strong hydrophobic and hydrophilic attractions, respectively. As a result, the detection sensitivity is improved by 2 orders of magnitude better than the use of the traditional MALDI plate, and high-quality mass spectra are obtained even in the presence of NaCl (1 M), NH4HCO3 (100 mM), or urea (1 M). The practical application of this method is further demonstrated by the successful analysis of myoglobin digests with high sequence coverage, demonstrating the great potential in proteomic research.
Aflatoxin B1-lysine is a metabolite produced by the liver after consumption of food contaminated with aflatoxin B1 (AFB1), a carcinogen produced by fungus of the genus Aspergillus, mainly A. flavus and A. parasiticus. Measurement of AFB1-lysine in the human or animal blood is a valuable biomarker of AFB1 exposure through the diet. AFB1-lysine standards are not commercially available. This work reports the preparation and purification of AFB1-lysine from AFB1-exo-8,9-epoxide obtained by epoxidation of AFB1 with chloroperoxybenzoic acid in dichloromethane and phosphate buffer. After formation of AFB1-dialdehyde in equilibrium with AFB1-diol, L-lysine was added and the reaction remained for 24 h to form AFB1-lysine. LC-MS/MS analysis was performed in positive ion mode and showed the parent molecular ion \([\text{M} + \text{H}]^+, \text{m/z} 457.1\) yielding a dominant fragment at \text{m/z} 394 (\text{M} + \text{H} - \text{NH}_3 - \text{CO} - \text{H}_2\text{O})\), and minor fragments at \text{m/z} 376, 366, 348 and 328. The spectrum obtained by the in-line diode-array detector was also consistent with that reported in literature. The NMR 1H of AFB1-lysine was performed in deuterium oxide (D2O) as solvent and NMR data was collected in an Avance DRX500 spectrometer system (500.13 MHz for 1H) at 300 K. Chemical shifts \(\delta\) obtained from the 1H NMR spectrum of AFB1-lysine synthesized were similar to data reported for AFB1-acetyl-lysine in the literature. In conclusion, preparation and purification of AFB1-lysine was completed in 24 h with 25% yield, being a simple and practical method that can be reproduced in analytical laboratories.
Nanoparticles can be successfully used for the creation of specific surface morphologies and the precise location of specific chemical functions at the surface. In this work, the local chemical surface composition of organic nanoparticle coatings including different vegetable oils was investigated by means of high-resolution and imaging ToF-SIMS. More interestingly, principal component analysis of the spectra after selection of specific mass m/z allowed for the classification of coated papers depending on the type of used nanoparticles. We specifically discuss the influence of various selected moieties m/z from the spectra on the classification of the coated paper surfaces, where the focus on either peaks related to the coating, paper or fillers is considered. The spectra of coated papers can be grouped according to the incorporation of pure nanoparticles, saturated oil, mono-unsaturated oil, poly-unsaturated oil or wax. The quality of the coated papers was finally evaluated by mapping of chemical components over a surface area of 250 x 250 µm². As such, the interactions between the coating and the cellulose fibers together with the analysis of surface defects could be further detailed. We mainly illustrate the effects of different surface coverage and the distribution of different functional groups over the coating surface: specific groups related to the various coating ingredients were identified and their in-/homogeneous distribution or separation over the surface was evaluated. Mainly, some coating types mask the influence of inorganic fillers while the presentation of inorganics at the surface still prevails for other coating types. The analysis learns that both positive and negative ion spectra deliver complementary information for specific moieties in the coating systems and the coating qualities can be described in detail after extensive data analysis.

**Keywords:** Surface Analysis, Time of Flight MS

**Application Code:** Materials Science

**Methodology Code:** Mass Spectrometry
Since field asymmetric ion mobility spectrometry, FAIMS or DMS, is a powerful tool for separating ion species in an atmospheric pressure, it’s been widely used as both a standalone ion mobility detector and a pre-filter of mass spectrometer. When a high-voltage asymmetric waveform at a radio frequency combined with a compensation DC voltage applied between two electrodes of a FAIMS device, only ion species with a specific mobility will pass through the electrodes. In the conventional configuration, the scanning time of the compensation voltage reduces the detection cycle; the scanning time and separating resolution has a trade-off relationship. We have developed a novel FAIMS device which can detect ion species with different compensation voltages simultaneously. Usage of semi-conductive electrode enables to create the different compensation DC voltage at each position, and very weak ion current of fA level can be detected with each channel of an array IonCCDTM detector [1]. We’ll explain evaluation results of the FAIMS device combined with an electrospray ion source.


**Keywords:** Instrumentation, Ion Selective Electrodes

**Application Code:** General Interest

**Methodology Code:** Mass Spectrometry
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.

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. 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. As a result, an optimized vacuum system with all necessary components, such as chambers, backing pumps, turbomolecular pumps, vacuum gauges and peripheral devices in introduced.

**Keywords:** GC-MS, ICP-MS, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry

**Application Code:** Other (Specify)

**Methodology Code:** Mass Spectrometry
The clinical use of the enediyne-type anti-tumor antibiotics is hindered by their high toxicity. Aromatic carbon-centered biradicals are the reactive intermediates of these drugs. However, little is known about the reactions of the biradical intermediates with biological molecules. In this study, the reactivity of four positively charged aromatic carbon-centered biradicals (meta-benzyne analogs) towards aliphatic and aromatic amino acids was studied in the gas phase by using Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometry and the “distonic ion approach”. Previous studies have demonstrated that H atom abstraction (radical reaction) and NH2 abstraction (nonradical nucleophilic addition-elimination reaction) dominate the reactions of related monoradicals with amino acids, and their reaction efficiencies increase with the electrophilicity (electron affinity, EA) of the radical site. In sharp contrast, the major reactions of biradicals with amino acids include 2H atom abstraction, NH3 abstraction, H2O abstraction, addition–CO2, addition–HCOOH, addition–COOH, and formation of a stable adduct. Three main factors control the reactivity of metabenzynes with amino acids. They are the energy required to distort the minimum energy dehydrocarbon atom separation to the separation of the transition state, the singlet-triplet (S-T) splitting at the separation of the transition state, and the vertical electron affinity (EA) of the radical sites at the separation of the transition state. Additionally, the structures of the amino acids also influence the reactivity. High-level quantum chemical calculations were employed to obtain insights into some of the reaction mechanisms.

Abstract Text

The clinical use of the enediyne-type anti-tumor antibiotics is hindered by their high toxicity. Aromatic carbon-centered biradicals are the reactive intermediates of these drugs. However, little is known about the reactions of the biradical intermediates with biological molecules. In this study, the reactivity of four positively charged aromatic carbon-centered biradicals (meta-benzyne analogs) towards aliphatic and aromatic amino acids was studied in the gas phase by using Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometry and the “distonic ion approach”. Previous studies have demonstrated that H atom abstraction (radical reaction) and NH2 abstraction (nonradical nucleophilic addition-elimination reaction) dominate the reactions of related monoradicals with amino acids, and their reaction efficiencies increase with the electrophilicity (electron affinity, EA) of the radical site. In sharp contrast, the major reactions of biradicals with amino acids include 2H atom abstraction, NH3 abstraction, H2O abstraction, addition–CO2, addition–HCOOH, addition–COOH, and formation of a stable adduct. Three main factors control the reactivity of metabenzynes with amino acids. They are the energy required to distort the minimum energy dehydrocarbon atom separation to the separation of the transition state, the singlet-triplet (S-T) splitting at the separation of the transition state, and the vertical electron affinity (EA) of the radical sites at the separation of the transition state. Additionally, the structures of the amino acids also influence the reactivity. High-level quantum chemical calculations were employed to obtain insights into some of the reaction mechanisms.
DESI-MS-imaging has become a widely known method for the rapid analysis of surfaces taking only a couple of seconds. In DESI-MS-imaging every pixel contains the whole spectral information of the m/z-scan range selected. Applying appropriate statistical tools such as principle component analysis (PCA) as well as multivariate curve resolution (MCR) considerably simplifies the workflow for identifying spatially important features within the hyperspectral data-set.

In this study the practicability of DESI-MS-imaging has been demonstrated for the analysis of aged paper samples. As a first step, partially oxidized as well as partially acetylated compounds originating from ageing were successfully detected on the paper surface for the first time. In particular the following phenomena were investigated more in detail: the occurrence of ageing products with respect to a) the type of ink present on the paper surface, b) the ageing conditions applied and c) the local distribution of chromophores close to ink applications.

In a second step, DESI-MS-imaging was combined with multivariate statistical tools for monitoring phenomena originating from paper degradation. So-called foxing-stains, which are producing brownish spots on paper surfaces, were investigated more in detail. For the first time spatially important features could be identified.

In summary, DESI-MS-imaging coupled with appropriate statistical tools proves to be a powerful technique for the rapid profiling of paper degradation. Currently we continue the development of DESI-MS methods for accessing the chemical information present on the paper surface.

Acknowledgements

The financial support of the Christian Doppler Research Society (Christian Doppler Laboratory: Advanced cellulose chemistry and analytics) is gratefully acknowledged.

Keywords: Chemometrics, Imaging, Mass Spectrometry, Surface Analysis
Application Code: Other (Specify)
Methodology Code: Mass Spectrometry
Tetracyclines (TCs) are a group of broad-spectrum antibiotics, produced by actinobacteria. TCs remain the treatment of choice for infections caused by Gram-negative bacteria, Gram-positive bacteria, chlamydia, rickettsia, brucellosis, and spirochetal infections. In animal husbandry, TCs were widely used in veterinary medicine, which pose potential threats to the environment. In this study, a quick and sensitive analytical method was developed for the determination of 7 TCs (minocycline, oxytetracyclin, tetracycline, demeclocycline, aureomycin, methacycline, doxycycline). Sample pretreatment was conducted by solid-phase extraction to enrich TCs from surface water. Analysis was performed in 5 minutes for a single run by ultra high performance liquid chromatography/electrospray tandem mass spectrometry. The calibration curves of 7 TCs were constructed over a concentration range of 1.0 - 500 µg/L with correlation coefficients better than 0.999. Good repeatability on both retention time (%RSD: 0.21%) and peak area (%RSD: 3.73%) was obtained by 6 consecutive injections of standard mixtures at 10, 50, and 100 µg/L, respectively. A method was successfully established for fast, sensitive and accurate quantitative determination of 7 TCs in surface water.
Desorption electrospray ionization mass spectrometry (DESI-MS) is a powerful method for generating ions at ambient conditions without the need for sample preparation or pretreatment. In DESI-MS, a continuous stream of charged microdroplets impacts a surface containing a sample (cDESI-MS). Upon impact, analyte molecules are extracted from the surface into secondary microdroplets, from which gas-phase ions are eventually formed. We have developed a pulsed DESI-MS source (pDESI-MS) that demonstrates higher sensitivity. In addition, the pDESI-MS source is designed so that desorption only occurs during ion accumulation time (IT) of an ion trap mass spectrometer, increasing the sampling efficiency (SE) closer to 100%. This capability is particularly advantageous for high-resolution instruments such as the LTQ-Orbitrap hybrid mass spectrometers that require significant transient acquisition times after ion accumulation (e.g. for IT = 0.5 s and m/z-resolution = 100,000, and scan time = ~2.3 s, the SE increases by a factor of ~5 from 22% to 100%). In addition, pulsing the primary microdroplet spray reduces the total volume of deposited solvent per m/z scan, which minimizes the ‘washing effect’ reported for cDESI-MS on surfaces such as glass or Teflon®. The ability to control the amount of deposited solvent by varying the pulse widths offer the potential for improved spatial resolution in imaging mode. These results demonstrate that pDESI-MS offers the potential to transform current approaches for implementing spray-based ambient ionization techniques in chemical analysis and imaging.

Keywords: Biological Samples, Imaging, Instrumentation, Mass Spectrometry

Application Code: General Interest

Methodology Code: Mass Spectrometry
Phosphorothioate pesticides are used for control of insects that feed on fruits and vegetables. Pesticide residues can be monitored using liquid chromatography and electrospray ionization mass spectrometry (ESI-MS). The objective of this study was to study the formation of charged complexes with Ag\([sup]+[/sup]\) and Cu\([sup]2+[/sup]\) and determine if formation of these complexes can improve the ESI-MS detection of phosphorothioate pesticides. Experiments were carried out in positive ion mode on a quadrupole ion trap mass spectrometer equipped with an electrospray ionization source. Phosphorothioate pesticides investigated included fenitrothion, parathion, malathion, and diazinon. We found that the signal-to-background ratios (S/B) of mass spectra acquired from pesticide solutions spiked with Cu\([sup]2+[/sup]\) or Ag\([sup]+[/sup]\) were respectively ~7 to ~35 times those of mass spectra acquired from unspiked samples. S/B of mass spectra acquired from solutions containing malathion and diazinon were twice that of fenitrothion and parathion for both Ag\([sup]+[/sup]\)- and Cu\([sup]2+[/sup]\)-spiked samples, possibly due to differences in complex stability. The relative affinity of each pesticide for Ag\([sup]+[/sup]\) was also investigated and was found to follow the order diazinon > malathion > fenitrothion > parathion. Our results show that signal enhancement resulting from Cu\([sup]2+[/sup]\) or Ag\([sup]+[/sup]\) complexation is significant. These results will be used in developing a desorption electrospray ionization MS (DESI-MS) technique for direct surface analysis of agricultural food products for phosphorothioate pesticides. The authors acknowledge the University of Idaho for funding.

**Keywords:** Analysis, Electrospray, Mass Spectrometry, Pesticides

**Application Code:** Agriculture

**Methodology Code:** Mass Spectrometry
Comprehensive two-dimensional gas chromatography (GCxGC) offers greatly enhanced peak capacity through the coupling of two columns of different selectivity. The coupling of this technique with time-of-flight mass spectrometry (GCxGC-TOF MS) provides the additional benefits of highly sensitive detection and definitive mass spectral identification of trace-level analytes, making it the ideal choice for petrochemical analyses.

Despite this additional separation capacity, it may still be difficult to identify individual compounds when similar mass spectral characteristics are evident across an entire chemical class. Branched alkanes are a prime example, and have weak molecular ions, which further complicates the identification process. Until now, the accepted solution to this problem was time-consuming analysis of multiple standards of individual species to establish associated retention times or retention indices.

Select-eV\(^{\text{\textregistered}}\) technology from Markes offers a quicker alternative approach to identifying spectrally similar compounds. Select-eV is a novel ion source technology which gives users the ability to switch between hard and soft electron ionisation with no inherent loss in sensitivity. This ground-breaking development offers a wide, tuneable range of ionisation energies with no requirement for source switching or additional reagent gases.

The ability to provide enhanced molecular ions whilst retaining larger, structurally-significant fragment ions helps to differentiate between isomeric compounds, allowing confident identification and quantitation without having to analyse multiple standards.

**Keywords:** GC-MS, Mass Spectrometry, Time of Flight MS

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

**Methodology Code:** Mass Spectrometry
An analytical method for the determination of multiple pesticide residues in animal foods by modified QuEChERS method with on-line gel permeation chromatography/gas chromatography/mass spectrometry (GPC/GC/MS). Targets were 21 pesticides: methamidophos, dichlorvos, \textit{BHC}, \textit{BHC}, \textit{BHC}, \textit{BHC}, dimethoate, terbufos, chlorpyrifos-methyl, metolachlor, isocarbophos, p, p'-DDE, p, p'-DDD, o, p'-DDT, p, p'-DDT, phentriazophos, permethrin, cypermethrin, flucythrinate, fenvalerate, and deltamethrin. The samples were extracted from homogenized foods with acetonitrile assisted by n-hexane, and separated with liquid-liquid partition. The supernatant liquid was purified by PSA and C18 to remove most of the fats and pigments in samples, then after on-line GPC-GC/MS analysis which further removed macromolecular interference material, such as fat, the background interference brought about by the complex matrix in samples was effectively reduced. At the spiked level of 0.02 mg/kg, recoveries for most of pesticides were from 72.9% to 118.6%, and the relative standard deviations ranged from 0.88% to 9.67%. The limit of detection and the limit of quantification were 0.6~2.5 \textmu g/kg and 2.0~8.4 \textmu g/kg, respectively. The method is simple, rapid and characterized with acceptable sensitivity and accuracy to meet the requirements for the analysis of multiple pesticide residues in animal foods.

Keywords: GC-MS, Mass Spectrometry, Pesticides, Sample Preparation

Application Code: Safety

Methodology Code: Mass Spectrometry
Dithiocarbamates are common fungicides widely used in agriculture. In mammals dithiocarbamates typically show low acute toxicity, but after prolonged exposure the toxicity significantly increases. The European Standard EN12396-2 describes a method for the determination of dithiocarbamate residues in non-fatty foods, based on the reaction with tin-(II)-chloride and hydrochloric acid and consequent release of CS2. The CS2 amount, proportional to the concentration of fungicides, is analyzed by GC coupled to electron capture or MS detector.

A new approach based on the use of Static Headspace technique coupled to GC-TOFMS is described for the determination of dithiocarbamate residues in fruit and vegetable samples, in compliance with the method EN12396-2. The sample is heated and shaken in presence of HCl and SnCl2 into a closed headspace vial and, after the incubation time, an aliquot of the headspace is automatically transferred to the GC-TOFMS system for CS2 analysis. The obtained results confirmed the effectiveness of the approach for fruit and vegetable extracts (pear and spinach). In the concentration range suggested by the EN12396-2, the method exhibited excellent linearity, assuring a reliable quantification in real samples. The SHS automation guaranties high precision with RSD<1.1%. A limit of detection of 0.2 ppb for CS2 was extrapolated considering a signal 3 times the noise: this limit corresponds to a concentration of 0,68 ug/Kg of dithiocarbamate pesticides in the sample. Besides, TOFMS allows to easily recognize and confirm CS2 peak according to the full mass spectrum conveniently matched with the NIST library.
The ability to accurately and precisely characterize the molar mass distribution and averages of synthetic and natural polymers is essential as the shape and the breadth of a polymer's molar mass distribution will dictate the end-use properties of the polymer. One of the most highly used tools for characterizing the molar mass of polymers is size exclusion chromatography (SEC). Here, we will show the multiple utilities of SEC along with the time and resource saving benefits of implementing a chromatography system with low dead volume, a dual-flow refractive index detector and semi-micro columns for fast and accurate characterization of polymers. We will demonstrate how single-detector SEC can be used to monitor the synthesis of PEGylated polymers to determine the oligomeric content, as an approach to failure analysis for synthetic polymers, and for the characterization of commercial rubbers. The molar mass distributions of these synthetic polymers was fully characterized in less than 20 minutes and provided significant pictorial and numerical data for the differentiation between products. Finally, we will show how SEC coupled to a train of detection methods can be used to provide a detail picture of molar mass and polymeric size of natural polymers. Through these multiple applications we will also demonstrate how a low dead volume SEC system equipped with a dual-flow refractive index detector and semi-micro columns will save time and resources when analyzing polymers.

Keywords: Liquid Chromatography, Method Development, Rubber, Separation Sciences
Application Code: Polymers and Plastics
Methodology Code: Separation Sciences
Polyacrylnitrile (PAN) is often used as precursor material for the production of carbon fibers. During pyrolysis, a huge amount of organic but also inorganic gaseous products were released. The new STA 449 Perseus coupled to a GC-MS is a very powerful tool for the characterization of these pyrolysis reactions. The FT-IR is direct coupled to the STA (Simultaneous Thermoanalyzer TG-DSC/DTA) and heated to 300°C (transfer tube and gas measuring cell). This allows for very fast detection of the FT-IR spectra of the gases. Especially gases like HF, NH3 etc., are easy to identify compared to MS results, where interpretation can cause difficulties due to reaction with the quartz glass capillary or because of overlapping mass numbers of the different gas species.

The simultaneous GC-MS coupling allows identification of larger organic species with the advantage of the GC separation. The combination of TG-DSC-FT-IR-GC-MS allows detection of mass changes, energetics, and identification of the evolved gases by FT-IR and GC-MS of one and the same sample. The FT-IR and GC-MS combination helps confirm results or complement each other.

TG-DSC-FT-IR-GC-MS results of a PAN sample demonstrate the advantages of that instrument combination for studying pyrolysis reactions of such kind of materials.

Keywords: FTIR, Gas Chromatography/Mass Spectrometry, Pyrolysis, Thermal Analysis
Application Code: Polymers and Plastics
Methodology Code: Thermal Analysis
A multiple technique approach has been developed for the identification of polymer additives. The first step in the process involves chloroform extraction of the additives from the polymer. The extract is analyzed using gas chromatography-mass spectrometry (GC-MS) followed by liquid chromatography-mass spectrometry (LC-MS). As many additives as possible are identified from the GC-MS analysis. An ion trap mass spectrometer is used for the LC-MS analysis, which affords multiple levels of fragmentation to assist in the identification of unknown compounds. An ultraviolet detector is coupled with the LC-MS instrument to identify compounds having UV chromophores and to confirm compounds have not been missed during the LC-MS analysis. The advantage of this combined GC-MS / LC-MS approach is often an additive is detected by one technique, but not the other. A second advantage of the process, the GC-MS data can be searched in spectral databases leading to the identification of many compounds that may also be detected by LC-MS. This leads to less data interpretation for the LC-MS data. Additional structural assignments are made using an internally generated LC-MS database and an internal database of known polymer additives. After identification of unknown compounds, quantitation of the additives can be achieved using the most appropriate instrument. Examples of this approach will be presented. Improvements for this approach and other applications of the process will also be discussed.

Keywords: Gas Chromatography/Mass Spectrometry, HPLC, Liquid Chromatography/Mass Spectroscopy, Polymers and Plastics
Application Code: Liquid Chromatography/Mass Spectrometry
Anti-biofouling materials have wide applications in biomedical device and marine industry. Recently, biocide modified poly(dimethylsiloxane) (PDMS) and hydrophilic zwitterionic polymer brushes have been developed and showed good anti-biofouling performances. However, such materials may have various chemical structures that change their properties in different environments. Since anti-biofouling capability depends on surface structures and such materials are usually used in water, we applied sum frequency generation (SFG) vibrational spectroscopy to study the surface structures of these new polymers. The restructuring behavior of the polymers in water and the water structure at the polymer interfaces were probed in situ. SFG results showed that in air, the methyl group of PDMS dominated the surface, while in water, the alkyl chain of the biocide on PDMS stuck out to kill organisms causing biofouling. For zwitterionic materials, the polymer brushes stretched with good ordering in water, whereas water formed stronger hydrogen bonding with polymer. In addition, water ordering is strongest when the polymer brushes showed best anti-biofouling properties.

Keywords: Infrared and Raman, Materials Science, Polymers & Plastics, Spectroscopy
Application Code: Polymers and Plastics
Methodology Code: Molecular Spectroscopy
The truly interesting and research worthy proteins are found in small concentrations in serum. Analysis of biofluids for potential biomarkers (i.e. proteins) has been of great interest in medicinal research; however, most biomarkers are typically found in minute concentrations and masked by more abundant proteins. A large portion of albumin hinders the collection of the proteins of interest. Our group has been developing novel stationary phases for the extraction of proteins via solid phase extraction (SPE). Capillary-channeled polymer (C-CP) can be made from nylon, polypropylene (PP), and polyester (PET) polymers. These polymers are melt-extruded so as to provide channels which run collinearly through the length of the fiber, increasing the available surface area. When pulled through an HPLC column, the fibers interdigitate to form tiny channels that run through the column. The fibers, when employed for the separation of macromolecules, are effectively non-porous. The polymeric structure of the fibers allows for reversed phase (RP), hydrophobic interactions (HIC) as well as ion exchange (IEX). With a focus on affinity chromatography, C-CP fibers can be functionalized in order to achieve specific separations. Cibacron Blue (CB) modified PEG-lipids functionalize PP fibers using hydrophobic interactions, leaving the functionalized head group to “hang” in the mobile phase to interact with the analyte of interest. With the CB dye’s affinity to albumin and the fibers’ efficient fluid transport, albumin is specifically depleted from serum.

Keywords: Bioanalytical, High Throughput Chemical Analysis, Separation Sciences, UV-VIS Absorbance/Luminescence

Application Code: Polymers and Plastics

Methodology Code: Separation Sciences
Polymer and Plastic Analysis

Analysis of Clear Finishes for Wood Using Pyrolysis-GC/MS

Clear finishes applied to protect wood surfaces, whether exterior wood, architectural surfaces, furniture, musical instruments or decorative items, generally produce insoluble polymeric coatings difficult to analyze using typical laboratory instruments. Natural oils such as Tung and Linseed are still used, but synthetics, especially polyurethanes, are perhaps the most common. These polyurethanes may involve a variety of diisocyanates and polyols, plus other constituents. Customers associate the term “polyurethane” with toughness and quality, so many products sold as polyurethanes may in fact use a variety of other ingredients with some polyurethane included to justify the name. Originally oil-based, new polyurethane formulations may also be water-based, introducing additional analytical considerations.

Analytical pyrolysis coupled with GC/MS provides a simple way to examine the nature of such polymeric finishes. Polyurethanes regenerate the diisocyanates used, including preserving the content and ratio of isomers. Other synthetics, such as polystyrene and acrylcs, are easily determined whether alone or in a complex copolymer formulation with other synthetic or natural film producing products. Figure 1 below shows a comparison of two polyurethane formulations, one with the addition of styrene and acrylic. Examples are given of natural, oil-based finishes such as Tung oil, plus a variety of interior and exterior contemporary clear finishes, both oil- and water-based.

Keywords: Paint/Coatings, Polymers & Plastics, Pyrolysis, Sample Introduction
Application Code: Polymers and Plastics
Methodology Code: Gas Chromatography/Mass Spectrometry
As part of safety risk assessment, it is very important to identify and quantify the plastics additives and degradants, as extractables and leachables, from plastic materials that are widely used in pharmaceutical and medical device manufacture and storage because these compounds may pose safety risks to patients and/or change the efficacy of the medical products. One way to estimate the completeness of the extractable/leachable analyses to account for all compounds is to measure the Total Organic Carbon (TOC) of the extracted solution and compare that with the combined TOC of all compounds analyzed by a variety of analytical methods. Since formic acid and acetic acid are common extractables/leachables from plastics, and usually contribute large percentages to TOC, it is important to accurately quantify them. However, they are not easily detected by commonly used GC and HPLC methods due to their highly polar nature.

A simple and rapid method using UHPLC with UV detection (210 nm) was developed to determine formic acid, acetic acid, and citric acid. A UHPLC C18 column (2.1 X 100 mm, 1.8 um, 100 Å) and a mobile phase containing a mixture of acetonitrile and 10 mM phosphoric acid (pH 2.3) were employed. Formic acid, acetic acid and citric acid were separated within 3.2 min. The method was precise, sensitive (LOD = 1 ppm) and linear (1-100 ppm). The results of the development, performance and applications of this method to extractable/leachable analyses of several medical device materials will be presented.

**Keywords:** HPLC, Liquid Chromatography, Materials Characterization, Polymers & Plastics

**Application Code:** Polymers and Plastics

**Methodology Code:** Liquid Chromatography
Polymerized poly(ethylene glycol) diacrylate (poly-PEGDA) was fabricated into pneumatically controlled, non-elastomeric membrane valves as a nonspecific adsorption resistant alternative to polydimethylsiloxane valves. Temporal response, valve closure, and long-term durability of these poly-PEGDA valves were evaluated. Valve operation up to 8 Hz was achieved with a ~100 ms opening time (without applied vacuum) and a ~20 ms closure time. Valve closure demonstrates less leakage in geometries with a larger membrane contact area (>0.3 mm²) and pedestals wider than 15 [micro]m. After ~1000 actuations to reconfigure polymer chains and increase membrane elasticity, the fluid pressure needed to open a closed valve becomes the same as the applied control pressure. After this initial conditioning, the poly-PEGDA valves maintain a linear relationship between the closure pressure and the opening pressure required to initiate flow even after 115,000 actuations. These robust valves have potential for application as peristaltic pumps and in small volume bioassays or impedance detection.

Funding was provided by the National Institutes of Health (R01 EB006124) and the Telford & Frank Woolley and Garth L. Lee Graduate Student Research Awards from the Department of Chemistry and Biochemistry at Brigham Young University.

Keywords: Lab-on-a-Chip/Microfluidics, Laboratory Automation, Polymers & Plastics
Application Code: Polymers and Plastics
Methodology Code: Microfluidics/Lab-on-a-Chip
Analytical pyrolysis has long been used in the study of complex solids such as polymers and fuel sources. These studies are generally carried out in an inert atmosphere — typically helium — with analysis by gas chromatography or GC/MS. The same instrumentation may be used to investigate the effect of heating the sample in hydrogen, but usually the pyrolysis reactions are so fast that the degree of hydrogenation is minimal. For quantitative hydrogenation, a two-stage approach is needed, first pyrolysis of the sample, followed by hydrogenation over a suitable catalyst in a separate reactor.

The system used for these samples includes a filament based pyrolysis instrument equipped with a separate, temperature controlled reactor capable of operating at ambient or elevated pressure. Products generated are collected onto a sorbent trap to depressurize, and then transferred thermally to a GC/MS. An example comparing pyrolysis in helium and in hydrogen with a catalyst is shown in Figure 1 below. The expanded chromatogram shows that when petrochemical sources are pyrolyzed, they produce both saturated and unsaturated hydrocarbons. The introduction of a platinum catalyst operated at mild conditions hydrogenates the olefins, converting the alkenes to alkanes. The same instrumentation may be used to study the structure of synthetic polymers as well, especially polyolefins. Hydrogen pressure may be varied from ambient to 500 PSI, and examples are shown of the effect of increased pressure on the production of pyrolysis products from samples like polyethylene, petrochemical sources and natural oils.

Keywords: Hydrocarbons, Petrochemical, Polymers & Plastics, Pyrolysis
Application Code: Polymers and Plastics
Methodology Code: Gas Chromatography/Mass Spectrometry
Various novel colored azo polymeric (Polyureas) have been prepared by using a variety of benzothiazole derivatives, m-phenylene diamine and hexamethylene diisocyanate. The resultant colored polyureas were characterized by elemental analysis, IR, thermogravimetry. All the Polyureas were applied on Polyester fabric. 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.
Surfaces with water repellent properties arise from a characteristic interplay between surface chemistry and topography \[1\]. Recently topology optimization has been used to calculate an optimal shape of surface structures to increase the performance of a super hydrophobic surface \[2\]. The purpose of the presented work is to experimentally show the effect of this topology optimization.

To achieve a super hydrophobic surface any droplet on the surface should be suspended by the surface structure in the so-called Cassie-Baxter state \[3\]. For many super hydrophobic surfaces the Cassie-Baxter state is not energetically favorable over the Wenzel state, but the transition between the states is inhibited by a sufficient energy barrier. Topology optimization has been used to calculate the optimal shape of surface structures to increase the energy barrier of this transition \[2\], by minimizing the deflection of the water air interface between structures (Figure 1).

The different structures have been fabricated in Si using UV-Lithography followed by D-RIE etching (Figure 2). After removal of the resist the silicon have been FDTS coated to achieve a Young contact angle of 109° \[masculine ordinal\]. To characterize the stability of the Cassie Baxter state series of drops have been dropped at the surface to study the impact event. To quantify the results the impact event has been studied with High speed camera, contact angle hysteresis measurements and contact angle measurements (Figure 2).


**Keywords:** Optimization, Statistical Data Analysis, Surface Analysis, Water

**Application Code:** Polymers and Plastics

**Methodology Code:** Surface Analysis/Imaging
Methylene chloride is widely used as an extraction solvent for trace analysis in pharmaceutical, environmental, food, and chemical industries. With instrumentation advances leading to ever-lower analyte detection limits, we have implemented a quality by design (QBD) approach to produce a grade of methylene chloride that will provide interference-free analysis. Achieving this level of solvent quality has required manufacturing improvements which include an allowable impurity profile for raw material, additional purification processes, stringent quality control measures, and packaging innovations resulting in extremely clean product for trace analytical work. With respect to the environmental industry, use of methylene chloride for extracting slightly water soluble organic constituents is reported in several EPA methods (EPA 500, 600 and 8000 series). To mimic the extraction of trace level analytes using ultrapure solvent, neat samples of methylene chloride were spiked with parts per trillion of lindane and the recovery of lindane was assessed with different concentration processes such as rotary evaporator, Kuderna-Danish (K-D) apparatus, and a combination of both. Gas chromatography hyphenated with various detector systems (GC-MS, GC-ECD, and GC-FID) found that ultrapure methylene chloride produced an interference-free baseline during trace analysis of standards such as lindane. Our QBD approach ensures the consistent production of ultrapure methylene chloride which meets all purity specifications not only “at the time of manufacturing” but when the analyst first opens the container.
Real-time monitoring of metal concentrations in liquid flows would be highly desirable in applications like environmental pollution monitoring, waste water treatment and industrial process control. Currently, no feasible instrumentation capable of real-time metal analysis down to sub-mg/L concentrations level is available. Here we present a spectrometer employing micro-plasma emission spectroscopy (MPES) for real-time determination of metal concentrations in liquid flows. MPES is a technique where a discharge is generated in a narrow liquid filled capillary and the concentration of the targeted atomic species is retrieved from the discrete lines of the emission spectrum. Traditionally, MPES has been applied to off-line studies where samples are first prepared and then pipetted into a static sample volume. Typical detection limits vary from ppm to ppb concentrations depending on the analyte. Results will be presented on real-time analysis of sodium, potassium, silver and lithium. A detection limit of 0.2 mg/L has been demonstrated for sodium in a measurement time of 3.2 seconds. In optimum operation conditions 1% signal stability has been reached.

Authors would like to acknowledge Pohjois-Pohjanmaa Council of Oulu Region and Finnish Funding Agency for Technology and Innovation (TEKES) for their financial support.

Keywords: Elemental Analysis, Lab-on-a-Chip/Microfluidics, Plasma, Spectroscopy
Application Code: Process Analytical Chemistry
Methodology Code: Atomic Spectroscopy/Elemental Analysis
A direct headspace analysis of a standard mixture of volatile organic compounds (VOCs) in methanol/water was conducted using a molecular rotational resonance (MRR) spectrometer. Purge and trap GC methods like EPA method 524 have inherent interference problems for low molecular weight components and high vapor pressure gases. They require an hour of laboratory time and an experienced chemist for analysis. MRR spectroscopy is a fundamentally new, high resolution detector for analytical chemistry suited for mixture analysis where interference in column chromatography is a problem. Sample preparation is simple. The solution mixtures were injected into an evacuated vial and the equilibrated headspace was sampled through a needle delivering it directly to the spectrometer measurement cell. No heating or sample pre-concentration methods were employed. Broadband library matching was used to identify each component in the headspace, and targeted sensing was used to establish detection limits and a dynamic range of $10^5$.

Detection limits for the more polar, lower molecular weight molecules are comparable to EPA method 524. For example, the chloromethane MRR detection limit is 0.1 g/L and is accomplished in 40 seconds. MRR results are obtained in 10 minutes for full band composition analysis or in 40 seconds per component for targeted analysis. This study has important implications for applying MRR in headspace measurements above reaction solutions for process monitoring of solvents.
We present new techniques for direct analysis of gas mixtures based on molecular rotational resonance (MRR), a new high resolution molecular spectroscopy technique for analytical chemistry applications. These measurements are enabled by the selectivity of pure rotational spectroscopy and the time resolved Fourier transform MRR design. While pure rotational spectroscopy is a well-established technique for structural characterization in academic research applications, the development of room-temperature, benchtop, Fourier transform instruments was only recently made feasible by advances in microwave and millimeter wave digital electronics. The pure rotational energy manifold of a gas phase molecule is governed by quantized angular momentum states dependent upon the moment of inertia. Any redistribution of mass in a molecule shifts the rotational spectrum, so isomers, conformers and isotopologues (with site specificity) are all distinguishable. The full bandwidth of the instrument, with ~10^5 distinct spectral resolution elements captures the highly resolved, periodic rotational fingerprints in the mixture and the components can be identified by simple library matching. For the unidentified (or ambiguous) lines in the spectrum, Fourier transform MRR can also be used to enhance the analysis through nutation dipole estimation, two-color pump-probe identity verification, and mass estimation measurements. Here we show the structural specificity, sensitivity, dynamic range, quantitation accuracy, and unknown identification capabilities of Fourier transform MRR spectroscopy.
Whether commercial production of a material is from organic syntheses, isolation from a natural biological material, or deliberate formulation of a solid mixture by incorporation of various added ingredients, this newly available technique has proven useful. In the cases cited, the quantitative analytical problem is not trivial, particularly when the end product is a mixture of solids. To avoid classical wet separation techniques, quantitative chemical imaging is worthy of consideration. The commercial value of a product is assured by determination of its purity from byproducts in the processed mixture and the efficiency of the workup of a synthetic material. In cases where physical separation is used to isolate the desired product (such as wheat endosperm) from other botanical parts, the completeness of separation may be determined by quantitative imaging of the solid and similarly in a formulation of a mixture. The size of the analyte particles determines the appropriate pixel size used for imaging. Limitations of spatial resolution have a bearing on the field of view used. Multiple fields of view may be required to have a representative sample. Examples of chemical imaging applied to various production methods and various solid mixtures is presented. Success of the recent past will open future prospects of applying this technique in industry.

Keywords: Imaging, Near Infrared, Process Analytical Chemistry, Quantitative
Characterization of reaction mechanism and kinetics provides necessary information during the drug development process. Comprehensive mechanistic understanding affords reaction variable control to minimize side reactions, guide impurity control, and optimize yield. This poster presents an in-situ NMR in tandem with on-line HPLC. Together, these complimentary techniques were utilized to achieve full mechanistic characterization of a cyclization reaction composed of unstable intermediates. Off-line HRMS provided additional confirmation of the proposed intermediates that were derived from the on-line and in-situ data sources. This technology and methodology is being routinely and robustly applied for reaction characterization.
Composting is becoming increasingly popular among communities and family homes because it allows for a way to reduce the waste that is being dumped into landfills while producing a useful product. Composts are made by decomposing organic material to make fertilizer that can be used for gardening, landscaping, or agriculture. Depending on the material that is added to the compost, harmful herbicides and pesticides can get into the composting fertilizer. Clopyralid is an ingredient in some herbicides that has been shown to accumulate in composts due to its slow degradation. This synthetic hormone can cause abnormalities in legumes, solanaceous and composite plant families, and may also have adverse side effects in humans and other mammals. This work will help several Rochester, New York communities determine if their composts contain high levels of this synthetic hormone. An initial extraction using HPLC water, involves agitation to dissolve and extract clopyralid from the compost soil. After extensive trials, water has been shown to be the most successful solvent at extracting the clopyralid from soil. The extracted sample will be subjected to solid phase extraction. Various SPE cartridges, including an Oasis HLB 6cc LP extraction cartridge and a Supelclean ENVI Florisil SPE cartridge, will be used to further clean and concentrate the original samples. Using GC-MS with an Agilent HP-5MS UI column (30m length, 0.250mm diameter, and 0.25µm Film), the identity and relative abundance of the clopyralid will be determined and compared to acceptable levels.

Keywords: Environmental/Soils, Extraction, Gas Chromatography/Mass Spectrometry, Solid Phase Extraction
Application Code: Process Analytical Chemistry
Methodology Code: Gas Chromatography/Mass Spectrometry
SERS Investigation of pH Effect on the Adsorption Behavior of 4-Carboxythiophenol on Ag Surface

4-carboxythiophenol (4-CTP) adsorbed on Ag colloids was investigated by surface-enhanced Raman scattering (SERS) spectroscopy. From the intensity variation of the symmetric stretching COO vibration band corresponding to different pH values, it shows that 4-CTP molecules are bonded to the Ag surface through the S atoms and are SERS-active as monolayer (Langmuir isotherm model) at acidic conditions, but only through the COO groups to show SERS activity with multilayer adsorption (Freundlich isotherm model) at neutral or alkaline conditions. Raman spectra calculated by density functional theory were compared with experimental observation to interpret the adsorption behavior of 4-CTP on Ag.
Introduction
Coal has been used for centuries as the major energy source. It is necessary to properly understand and characterize the use of coal and its derivative products, such as ash and fly ash, in order to reduce their environmental impact.

Another advantage of the characterization of coal is the prevention of erosion and corrosion on industrial equipment caused by the mineral deposits in the furnaces.

Standard methods including the ASTM D6349-11 and the AS 1038.14.1-2003 use ICP-OES or AAS for this type of characterization. In the ASTM D6357-09, ICP-MS or graphite furnace are the alternative analytical methods used for low concentrations.

The purpose of this project is to demonstrate that the accuracy and precision mentioned in these standard methods can be achieved when using automated borate fusions as a method of dissolution for analysis by ICP-OES.

Materials and Methods
Three different samples and three reference materials were tested: NCS FC28127 – (Coal), VS-7177-95 (Coal ash) and EOP 12-1-02 (Fly ash).

Each solution was prepared in less than 12 minutes on a 3 position automated Fluxer using lithium metaborate flux.

Quantitative analyses were performed using an ICP-OES. All calibration standards were matrix matched.

Significance
The significance of this research resides in the simplicity of the method, its speed, automation and the complete absence of harsh acids.

Keywords: Coal, Elemental Analysis, ICP, Sample Preparation
Application Code: Process Analytical Chemistry
Methodology Code: Sampling and Sample Preparation
Spectrophotometric Determination of Copper Using 2-Hydroxy-4-Isobutoxy Acetophenone Oxime

2-Hydroxy-4-isobutoxy acetophenone oxime (HIBAO) has been used for the spectrophotometric determination for Cu(II) at pH range 7 to 9 in chloroform medium. Job’s method for continuous variation, Yoe and Jones’ mole ratio method, the slope ratio method show metal ligand ratio in complex to be 1:2. The stability constant of the complex is found to be $4.32 \times 10^8$. The dark green coloured complex obeys Beer’s law in the concentration range 31.77 to 444.78 ppm for Cu(II) ion, while the optimum concentration range from Ringbom plot is found to be 127.08 to 381.24 ppm. The photometric sensitivity and molar absorptivity at the 650nm are found to be 0.488µg/cm² and 130 l mol⁻¹cm⁻¹ respectively. The standard free energy of formation of complex is -12.00 kcal/mole at 30°C. The complex is stable for 48 hrs. The reagent has also been found to give quite satisfactory results for Cu(II) present in alloy like brass, bronze and synthetic mixtures. The antimicrobial Activity of HIBAO and Cu-HIBAO complex have also been checked.

Keywords: Analysis, Spectrophotometry, Trace Analysis, UV-VIS Absorbance/Luminescence

Application Code: Process Analytical Chemistry

Methodology Code: UV/VIS
We demonstrate the usefulness of surface-enhanced Raman scattering (SERS) for determining values of critical micelle concentration (cmc) of cationic surfactants. In this study, cetyltrimethyl ammonium bromide (CTAB) and dodecyltrimethylammonium bromide (DoTAB) were selected as cationic surfactants and sodium dodecyl sulfate (SDS) was chosen as a control. The characteristic Raman bands of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB)-functionalized silver nanoparticles were monitored as a function of surfactant concentration. The working curve was obtained by plotting the SERS signal intensity ratio between the aromatic ring vibration at 1,558 cm⁻¹ and the symmetric nitro stretching of DTNB at 1,333 cm⁻¹ versus the concentration of cationic surfactants, a dramatic change in slope at a particular concentration could be used to determine the cmc of this surfactant. The cmc values for CTAB and DoTAB obtained under conditions are in good agreement with those reported in the literature.

Keywords: Process Analytical Chemistry, Spectroscopy, Surface Enhanced Raman, UV-VIS Absorbance/Luminescence
Application Code: Process Analytical Chemistry
Methodology Code: Vibrational Spectroscopy
2-Hydroxy-4-Isobutox-5-Bromo Acetophenone Thiosemicarbazone (Hibbat) has been used for the spectrophotometric determination for Cu(II) at pH range 3 to 10 in chloroform medium. Jobs’ method for continuous variation, Yoe and Jones’ mole ratio method, the slope ratio method show metal ligand ratio in complex to be 1:2. The stability constant of the complex is found to be $4.38 \times 10^8$. The green coloured complex obeys Beer’s law in the concentration range 15.89 to 111.20ppm for Cu(II) ion, while the optimum concentration range from Ringbom plot is found to be 15.89 to 79.43ppm. The photometric sensitivity and molar absorptivity at the 430 nm are found to be 0.143µg/cm² and 445 mol-1cm-1 respectively. The standard free energy of formation of complex is -$11.98$ kcal/mole at 30°C. The complex is stable for 48 hrs and up to 45°C in chloroform. The reagent has also been found to give quite satisfactory results for Cu(II) present in alloy like brass and bronze.

Abstract Text

Keywords: Analysis, Spectrophotometry, Trace Analysis, UV-VIS Absorbance/Luminescence
Application Code: Process Analytical Chemistry
Methodology Code: UV/VIS
Paper-based ion-selective electrodes (ISEs) are simple, flexible, and cost-efficient in comparison with conventional solid-contact ISEs. Yet, paper-based ISEs have poor limits of detection (in the micromolar range) relative to conventional solid-contact ISEs. Here we describe the construction and optimization of ultrasensitive ISEs based on commercially-available filter paper modified with single-walled carbon nanotubes, sputtered gold, and conductive polymer poly(3-octylthiophene) to support an ion-selective membrane. The ion-selective membrane is based on the copolymer methyl methacrylate-decyl methacrylate (MMA-DMA) and other components such as an ionophore and ionic sites. The copolymer MMA-DMA is highly water-repellent and has a low coefficient of diffusion, which makes it particularly suitable for the creation of sensors with high performance in reaching low limits of detection. Three different configurations of the electrodes have been characterized by using contact angle surface analysis, oxygen influence testing, and water layer testing. Ultrasensitive paper-strip ISEs for cadmium, silver, and potassium ions were constructed with groundbreaking limits of detection of $2.5 \times 10^{-9}$, $2.7 \times 10^{-8}$, and $3.3 \times 10^{-8}$ M, respectively. In addition to such low limits of detection, paper-strip ISEs display good selectivity and reproducibility and are flexible, allowing the possibility of applications of [i]in-situ[/i] analysis of bodily fluids (sweat, tears, etc.). These electrodes may also be applied in paper-based assays of biomolecules (proteins and DNA), offering cost-effective medical diagnostics.

The authors acknowledge the College of Science and the Department of Chemistry at University of Central Florida for financial support of this research.

**Keywords:** Electrochemistry, Ion Selective Electrodes, Potentiometry, Ultratrace Analysis

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
Complexation of Silver Ions by Natural Organic Matter as Studied Using Fluorous-Phase Ion-Selective Electrodes

Silver nanoparticles (AgNPs) are used extensively in both the medical and consumer industry as antimicrobial agents. The antibacterial nature of AgNPs is primarily due to oxidative dissolution of these particles and release of Ag\(^+\), which can bind to proteins, enzymes and DNA and disrupt normal cell functions. Therefore, it is critical to understand the extent and kinetics of dissolution to Ag\(^+\) and the environmental effects of the released Ag\(^+\) to understand AgNP toxicity. Natural organic matter, NOM, which originates from living organisms and is abundant in surface and ground water supplies, can interact with the released Ag\(^+\), resulting in silver ion speciation, decreased bioavailability, and altered toxicity. Therefore, understanding the chemical equilibria between Ag\(^+\) and NOM is critical for a correct assessment of toxicity and the environmental impact of AgNPs.

Ion-selective electrodes (ISEs) provide sensitive and selective measurements of Ag\(^+\) and permit the distinction between free silver ions and silver complexes in the solution. However, interference of biological moleculesshortens the life-time of the conventional ISEs and limits their application in real-life samples such as samples containing NOM. Fluorous-phase ISEs are an appealing tool for studying the interaction of NOM with Ag\(^+\) because they exploit the low polarity and polarizability of fluorous phases to expand the sensor selectivity range and to overcome problems encountered due to biofouling. In contradiction to hypotheses reported in prior studies, our findings suggest no significant binding between Ag\(^+\) and NOM under environmentally relevant conditions. It was found that at basic pH, weak complexation between NOM and Ag\(^+\) can be observed whereas at acidic and neutral pH there is no significant binding of Ag\(^+\) to NOM.


Keywords: Bioanalytical, Electrochemistry, Environmental/Water
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Sampling ultra-small volumes of liquids for analysis is essential in a number of fields from cell biology to microfluidics to nanotechnology and electrochemical energy storage. Here we demonstrate the possibility of employing chemical vapor deposition to deposit a layer of carbon on the inner wall of nanometer-sized quartz pipettes. This device can be used for sampling attoliter-to-picoliter volumes of fluids and determining redox species by voltammetry and coulometry. Very fast mass-transport within the carbon-coated nanocavity allows for rapid exhaustive electrolysis of the sampled material. The voltammogram consists of both symmetric current peaks resulting from the complete oxidation/reduction of redox species initially sampled inside the cavity and a steady-state current plateau produced by the diffusion of molecules from the bulk solution to the nanometer-sized orifice. A carbon pipette can be used as the tip in the scanning electrochemical microscope (SECM) to be precisely positioned at the sampling location. The developed device is potentially useful for solution sampling from biological cells, micropores and other microscopic objects.

The support of this work by the National Science Foundation (CHE-0957313) is gratefully acknowledged.
From the viewpoint of clinical analysis determination of urea in samples is an important task. Sensors allowing fast, reliable and cheap method of determination, especially disposable ones are required. One of possible approaches is application of urease as bio-receptor. In the presence of the enzyme – urea hydrolysis is observed resulting ultimately in pH change. Thus it possible to determine urea looking at potential change of pH sensitive potentiometric transducer. This work looks at possibility of application of CNTs based disposable potentiometric sensors for this purpose using ion-selective membranes sensitive to ammonium or hydrogen ions coated on CNTs layer.

Keywords: Bioanalytical, Biosensors, Potentiometry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Safranin O (SO, also known as dimethyl safranin or basic red 2) is a positively charged phenazine. SO was widely used in the textile industry. More recently, it has been used as a contrast stain to detect cytoplasm and nucleus of pathogens or microorganisms and to identify degenerative processes such as osteoarthritis. The latter is realized by binding to glycosaminoglycans (GAGs), which are abundant in the extracellular matrix of articular cartilage. In this study, SO was studied in media with a wide range of pH by voltammetry, spectrophotometry, and fluorometry. The carbon electrode was used to characterize the SO redox behavior. SO exhibits excellent reversible voltammetric property in aqueous media. SO also possesses active spectrofluorimetric behavior. In addition to SO's well-defined pH responding feature, we found that nitrite yields significant spectrophotometric, fluoroscopic, and voltammetric response that could be further enhanced by the presence of other oxidants. Moreover, these responses shed promise being a future sensitive method for this analyte in environmental samples. The potential co-existing material or interferents, including amino acids, sugars, small organic compounds, do not interfere with the nitrite determination. Mechanistic study showed a possible diazotation of safranin O accounts for the formation of responsive products in acidic media.

This work was supported by the NASA-TSGC-NIP, Texas TWC Workforce grant, NOAA-Watershed Grant, MSEIP Fund, and Welch Foundation departmental grant (BJ-0037).

Keywords: Electrochemistry, Environmental, Fluorescence, UV-VIS Absorbance/Luminescence
Application Code: Environmental
Methodology Code: Chemical Methods
Hydrophilic ion-exchange membranes with a high ion exchange capacity have shown interesting properties when used as sensing membranes of ion-selective electrodes (ISEs). Previous researchers demonstrated that such membranes exhibit Nernstian responses to different anions such as chloride, and it was pointed out that the hydrophilic nature of these membranes may be beneficial in view of biofouling caused by electrically neutral, hydrophobic interferents.1 While co-ion failure (i.e., Donnan failure) of hydrophilic ion-exchangers has been commented in the literature in the context of non-potentiometric applications, Donnan failure of ISEs based on hydrophilic ion-exchanger membranes has not yet been thoroughly studied. The purpose of this work is to compare the characteristics of Donnan failure of hydrophilic, high capacity ion-exchanger membranes with those of conventional ISEs with poly(vinylchloride) (PVC) membranes. Preliminary results indicate that the use of hydrophilic ion-exchange membranes reduces Donnan failure associated with very hydrophobic co-ions, which would be a substantial benefit for measurements in samples of biological origin.


Keywords: Ion Selective Electrodes
Application Code: Clinical/Toxicology
Methodology Code: Electrochemistry
Interfacial Electron Transfer Kinetics across Single Layer Graphene

Single layer graphene (SLG), as a new generation carbon material has attracted a great deal of attention recent years. The two dimensional, one atom layer graphene exhibits exceptional electrical and mechanical properties. SLG is electrochemically active and due to its extreme thinness, it can be used as an ideal platform to explore electron transfer processes across dissimilar environments. Here, we studied the electron transfer behavior of redox mediators across a SLG interface suspended at the interface between two immiscible electrolytes. Scanning electrochemical microscopy (SECM) was used to characterize with high accuracy and spatial resolution the redox properties of this interface. Previous studies have used SECM to characterize the apparent standard electron transfer rate constant of different mediators on graphene electrode surface in both aqueous and nonaqueous systems.

Large-scale, single-layer graphene were synthesized by chemical vapor deposition (CVD) method. By transferring graphene onto patterned polydimethylsiloxane (PDMS) micro-channel, we were able to suspend graphene at the interface of two different solutions below (in PDMS channel) and above graphene sheet. After introducing two different redox mediators in separate phase, the interfacial electron transfer across graphene was probed by SECM. This molecularly thin interface can be used to facilitate charge injection for redox species that decompose in aqueous solution or to couple electron transfer processes to ion transfer across liquid-liquid interfaces.

Keywords: Electrochemistry, Electrodes, Materials Science
Application Code: Materials Science
Methodology Code: Electrochemistry
Single layer graphene is the thinnest electrode available. It is virtually transparent, conducting and electrochemically active. These properties and its ability to be modified make it an ideal platform for exploring electrochemically-stimulated luminescence. This study utilizes single layer graphene for producing electrogenerated chemiluminescence (ECL) at both graphene and metal nanostructures deposited on it. ECL from highly-emitting systems such as tris(bipyridine)ruthenium(II) (Rubpy), 9, 10-diphenylanthracene (DPA), rubrene (RU) and BODIPY dyes were all carried out under inert and dry conditions in organic solvents. Scanning Electrochemical Microscopy (SECM) was used to induce steady state ECL emission in the diffusion region between the tip and the graphene substrate. Light was quantified with a photodetector beneath the sample with minimal optical interference from the substrate. The use of graphene additionally provides a unique platform for the study of different materials as it can be modified by nanoparticle deposition or patterning via lithography. The combination SECM-ECL study allows the analysis of differences in reactivity at different sites via electrochemical current and light emission. Significant work was also completed to extend the lifetime of the molecularly-thin graphene electrodes. Prior to use, graphene was coated in polytetrafluoroethylene leaving a window of exposed graphene on bare glass for electrochemical experiments, as shown in figure 1. This “protected graphene” shows significant improvement in the lifetime of the electrodes and paves the road to exciting applications in the nanopatterning of this extremely thin material.
**Session Title**  
SEAC: Society for Electroanalytical Chemistry Poster Session

**Abstract Title**  
Study of Degradation of Bimetallic Nanoparticle Electrocatalysts Using Micro-ITIES Interfaces as SECM Probes

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**Abstract Text**

Time- and electrode potential-dependent degradation processes of electrocatalysts and supports in fuel cell electrodes during operation lead to decreased device performance and sometimes failure. Here, scanning electrochemical microscopy (SECM) with versatile redox and ion-sensitive probes is used to investigate degradation processes at bimetallic electrocatalyst particles in combinatorial arrays during the oxygen reduction reaction (ORR) and related reactions. SECM using metallic micro- and nano-probes can be used to image and quantify the activity of nanoparticles deposited on an inert substrate. Similarly, pipette-suspended microscale interfaces at two immiscible electrolyte solutions (µ-ITIES), i.e. using a polarizable aqueous/organic interface, can be used to detect metal dissolution processes. Here, ligands in the organic phase coordinate dissolved metal ions in the aqueous phase, resulting in a facilitated ion transfer (FIT) of the metal ions into the organic phase, producing an ion current. At the µ-ITIES, chronoamperometry was used to quantify metal ion concentrations with spatial and temporal resolution.

Arrays of bimetallic nanoparticles produced through the cathodic corrosion method were prepared by micro-dispensing onto an inert glassy carbon electrode and characterizing their reactivity towards the ORR. µ-ITIES SECM probe was used for the in-situ characterization of these combinatorial arrays of bimetallic nanoparticles of platinum and various non-noble metals. This was done by operating the SECM in tip collection/substrate generation mode. These µ-ITIES were used to study dissolution kinetics of non-noble metals during electrocatalysis by positioning the interface near the nanoparticle array, biasing the array to catalytically active potentials, and monitoring the ion current during prolonged catalysis.

**Keywords:** Characterization, Electrochemistry, Metals, Microelectrode  
**Application Code:** Fuels, Energy and Petrochemical  
**Methodology Code:** Electrochemistry
Investigation and Characterization of Potentiometric-Scanning Ion Conductance Microscopy

Scanned probe microscopy (SPM) resolution is a complex issue dependent upon factors such as probe geometry and probe-surface interaction. Potentiometric-scanning ion conductance microscopy (P-SICM) is a recently described SPM technique based on scanning ion conductance microscopy (SICM). P-SICM resolution has not yet been investigated, but characterization of this technique's imaging capabilities has important implications for cellular studies already performed. To investigate P-SICM resolution, SICM resolution was first experimentally examined with nanoparticles. For P-SICM studies, a focused ion beam (FIB) was utilized to mill shapes with controlled dimensions in a silicon nitride membrane. In the future, the conductance of specific cellular features will be mimicked to serve as a direct comparison for substrate-specific resolution studies.

Keywords: Electrochemistry, Imaging, Instrumentation, Microscopy
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Ion-selective electrodes (ISEs) with Zn(II) tetraphenylporphyrin as receptor resulted in significantly improved selectivities for CN\(^{-}\). However, Zn(II) tetraphenylporphyrin reacts with molecular oxygen upon illumination to give singlet oxygen, a reactive oxidizing species that may initiate the opening of the porphyrin ring. UV-Vis spectroscopy showed the absorption peak shifted when membranes with Zn(II) tetraphenylporphyrin were exposed to light, indicating a decomposition of the ionophore. Moreover, the Zn(II) tetraphenylporphyrin based CN\(^{-}\)-selective electrodes also suffered from sub-Nernstian responses to CN\(^{-}\) and decreases in selectivities. However, no decreases in performance were found if the experiments were performed in a dark room. Furthermore, initial results showed that a Nernstian response to CN\(^{-}\) was maintained when the electrodes were protected with a 660 nm high pass filter while a loss of the Nernstian response was observed with 500 nm or 400 nm high pass filters. This observation suggests that the light sensitivity is caused directly by absorption of visible light by Zn(II) tetraphenylporphyrin, which exhibits absorption peaks at 425 nm, 550 nm and 640 nm.

A wide variety of light absorbing materials (e.g., graphite, carbon nanotubes, fullerene and different types of organic dyes) were incorporated into the ISE membranes in an attempt to prevent the degradation of the Zn(II) tetraphenylporphyrin. Membranes with single wall nanotube (SWNT) maintained Nernstian slope after 24 hours of light exposure while membranes without the SWNTs exhibited a decreased slope of 46 mV per decade. UV-Visible spectroscopy showed that only the membranes with SWNTs absorbs all the light from 400 nm and 700 nm, which is the damaging absorbance zone for Zn(II) tetraphenylporphyrin.


Keywords: Analysis, Electrodes, Environmental, Ion Selective Electrodes
Application Code: Environmental
Methodology Code: Electrochemistry
Development of Novel Cations to Extend the Electrochemical Window of Ionic Liquids: Improving the Energy Density of Nanostructured Supercapacitors for Electrical Energy Storage

Without significant risk and irreversible damage to the environment, the increasing global demand for energy can only be met by utilization of renewable energy resources. In this context, it is important that recent advances in the design of nanostructured materials have resulted in substantial increases in the energy density of supercapacitors, making them very attractive for energy storage applications. 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, is highly desirable.

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. Cations with saturated, straight or cyclic alkyl substituents attached to a nitrogen atom (as is the case, e.g., for tetraalkylammonium cations) are the most electrochemically stable cations. It was hypothesized that bulkier substituents attached to the cationic center would reduce the accessibility of the cationic center, extending the lower end of the electrochemical window. To investigate this effect, a comprehensive series of symmetrical and unsymmetrical quaternary ammoniums with varied steric hindrance was synthesized and the lower end of the electrochemical window was systematically analyzed using 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. Findings show that unlike hypothesized in many studies, steric hindrance does not affect the electrochemical stability of quaternary ammonium cations. This suggests that attempts to widen the potential window of quaternary ammonium cations should consider electronic effects, which have in the past been largely ignored when considering the electrochemical stability of quaternary ammonium ions.


Keywords: Electrochemistry, Energy, Voltammetry
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Electrochemistry
It has been previously shown that ion-selective electrodes (ISEs) with fluorous membranes have improved selectivity and an improved resistance to biofouling when compared to those with conventional 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 [1,2]. To test the possibility of eliminating the plasticizer and the need for solvents in the device fabrication, we have recently explored alternative fluoropolymers. In this work, ion-exchanger electrodes and silver ISEs have been fabricated using new highly fluorinated polymers and several silver ionophores. By using low glass transition temperature fluorous polymers, such as poly[(1H,1H,2H,2H-perfluorooctan-1-yl)styrene], the need for a plasticizer is removed. These electrodes show a Nernstian response and are highly selective for silver, comparable to some of the most selective silver ISEs. By synthesizing block copolymer or cross-linked versions of these polymers, a self-supported fluorous membrane ISE could be developed, increasing the applicability of fluorous membrane sensors by improving mechanical stability and an ability to be miniaturized compared to that of previous fluorous membrane ISEs.


This research has been supported by the National Science Foundation through grant OISE CHE-0809328.
Biofouling of Ion-Selective Electrode Membranes: The Role of Ionic Site Leaching into Biological Samples

The primary focus of this research is the investigation of the mechanism of biofouling in current fluorous phase ion-selective electrode (ISE) systems. With prolonged exposure to biological samples, potentiometric measurements using conventional polymeric-membrane ISEs exhibit a breakdown of selectivity and response. Therefore, extensive washing procedures and frequent recalibrations are needed for many clinical and biological applications. Initial work with fluorous phase ISEs has shown significant improvements in selectivity and limits of detection over conventional polymeric-membrane ISEs.\[^1\] Moreover, experiments with fluorous pH electrodes have shown that long term serum exposure does not affect the electrode selectivity but stirred serum solutions appeared to cause a transient EMF drift. To explore this effect more systematically, a potentiometric stir test was developed. Both conventional polymeric membrane and fluorous membrane electrodes were exposed to solutions stirred intermittently. Both types of membranes exhibited an EMF response to stirring when exposed to 10% v/v solutions equine blood serum but not when exposed to simple electrolyte solutions. The transient potentiometric response depends on the hydrophobicity of the ionic sites incorporated into the ISE membranes; specifically, a lower hydrophobicity results in a larger effect of stirring on the EMF.

For the fluorous electrodes, synthesis of a more hydrophobic ionic site and its use along with fluorophilic H[^+\sup\sup\]\[^\sup\sup\] ionophores successfully mitigated the effect of sample stirring on the EMF. The use of a fairly simple phase boundary model confirms that the effect of stirring is caused by loss of ionic sites into the serum-containing sample. While it has been known for a long time that the lifetime of ISEs in biological samples depends on the hydrophobicity of the ionophore and ionic sites, this is, to the best of our knowledge, the first report that shows that the leaching of ionic sites into serum samples can be directly observed by potentiometric monitoring of the effect of sample stirring on the EMF.


**Keywords:** Biological Samples, Ion Selective Electrodes, Membrane, Sensors

**Application Code:** Bioanalytical

**Methodology Code:** Electrochemistry
Capillary electrophoresis has evolved into a powerful tool for the control and analysis of biological samples. Compared with two-dimensional gel electrophoresis, 2D-CE has the advantages of low sample volume, quick analysis time, online detection, and ease of automation with mass spectrometry. It also has possibilities for a wide range of separation schemes considering the different modes of CE available. The main challenge in developing two-dimensional CE is in the design of an interface that can couple two orthogonal separation modes. In this report, we present an improved interface for 2D-CE automation and separation of complex biological mixtures. The performance of the new interface is demonstrated by comparing transfer efficiencies between a traditional 2D-CE interface and the new, nicked sleeve capillary 2D-CE interface. The developed interface is currently in use for studying the extent of protein phosphorylation.
Prednisolone acetate is a synthetically produced steroid hormone and is used widely in medical applications as an anti-inflammatory and immunosuppressant. A stability-indicating reversed-phase liquid chromatography method has been successfully developed that can accurately quantitate prednisolone acetate in prednisolone raw material and drug product. The stability-indicating capability of the method was demonstrated by adequate separation of prednisolone acetate and the potential degradation peaks with the use of an Agilent 1100 Series Liquid Chromatographic System when acidic, basic, oxidative, thermal and UV stresses were applied.

Chromatographic separation of prednisolone acetate and its degradation products were achieved under isocratic elution conditions at a flow rate of 1.0 mL/min. The organic solvent of choice was ACN and the mobile phase was optimized at a composition of 30% ACN and 70% buffered deionized water. The best suited buffer was a monobasic potassium phosphate salt with a concentration of 25 mM adjusted at pH 2.9.

The separation was performed on a reversed phase, silica based, Nova-Pak C18 column (150 x 3.9 mm) made by Waters®, with a particle size of 4 µm. The column temperature was controlled at 25º C and the UV absorption wavelength was set at 245 nm. The injected volume of prednisolone acetate was 10 µl and the total run time for the method was 25 minutes. The method was validated within ICH and FDA guidelines by establishing system suitability, specificity, robustness, solution stability, method precision, injection precision, intermediate precision, linearity, accuracy, limit of quantitation and limit of detection.
Abstract Text

Coumarin is naturally occurs flavoring substance in cinnamon and many other plants. For its strong fragrant odor, coumarin is widely used in industry as fragrance ingredient, found in 57 % of 73 deodorants on European market, and also in medicines due to bronchial dilator, anti-inflammatory and analgesic properties. Coumarin and its derivatives have also been evaluated for cancer treatment and HIV inhibitors. Vanilla flavoring is one of the most popular flavorings in the world. Artificial vanilla extract are generally less complex and can contain vanillin, ethyl vanillin and other related compounds. Recently, subcritical water chromatography has gained increasing attention due to organic solvent are not required in the subcritical water chromatography mobile phase and hence eliminated. In this study, we investigated the potential application of SBWC method for separation of coumarin, vanillin and ethyl vanillin. Separation columns used in this study include a Waters X-Terra MS C18, a Waters X-Bridge C18, a Zorbax RRHD Eclipse Plus, a Zorbax-SB Phenyl, a Zorbax-SB C18 column.

Results, the method developed confirmed that pure water can be employed as eluent to wholly separate coumarin’s at high column temperatures (100-200 oC) on five different commercial HPLC column (Fig 1). All worked column have been shown to be a suitable stationary phase for subcritical water chromatography with a good thermal stability.


Acknowledgements

This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK): grant number KBAG-112T336.

Keywords: Bioanalytical, High Temperature, HPLC, Pharmaceutical

Application Code: Bioanalytical

Methodology Code: Separation Sciences
Coumarin (1,2-benzopyrone), firstly isolated in 1822 from Tonka beans and it is the parent compound of a large class of naturally occurring phenolic constituents having fused benzene and pyrone rings (1). Coumarin is plant flavonoids widely distributed in nature. Natural coumarins are known to have anti-diabetic activity, anabolic antioxidant and hepato protective activities. Substituted coumarins derivatives have been reported to have variety of biological activities (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.

The purpose of this study is to determine the selectivity of the Na-PHEMA stationary phases and then to utilize them for the separation of various coumarin derivates by using HTLC. In addition we also demonstrated the stability of the NA-PHEMA phases using as a stationary phases compared to a typical stationary phase based on silica based stationary phase.

References

Acknowledgements

This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK): grant number KBAG-112T336.
Diabetes is a health condition that affects 25.8 million people in the United States and 366 million people worldwide. Diabetes is a metabolic disease related to insulin deficiency or glucose intolerance, which can result in elevated levels of glucose leading to non-enzymatic glycation of proteins. The purpose of this study was to characterize the metabolic effects of diabetes on the structure and function of serum transport proteins such as human serum albumin (HSA). High-performance affinity chromatography (HPAC) was used to examine the binding of different sulfonylurea drugs to HSA at various stages of glycation. Results have indicated a variation in the binding affinity for these drugs to HSA at different levels of glycation for both in vitro and in vivo glycated HSA samples. Mass spectrometry (MS) experiments through the use of matrix-assisted laser desorption/ionization time-of-flight MS and nano-electrospray ionization time-of-flight MS and MS/MS were used to obtain qualitative and quantitative structural information about the glycation modifications that occur on both in vitro and in vivo glycated HSA. These studies have resulted in the identification and location of glycation related structural modifications on HSA. The results from these studies can provide a better understanding of the metabolic effects of diabetes on drug-binding with HSA and protein glycation, which could be used in the future to develop improved treatment regimens for patients with diabetes.

Keywords: Bioanalytical, HPLC, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Separation Sciences
Protein analysis remains a difficult challenge in many fields of medical diagnosis and biochemical research. Large scale methods reach limitations especially for small volume samples and concomitantly low protein concentrations. In this regard, microfluidic devices offer several advantages over the larger counterparts, for instance efficiency, speed, and high throughput.

Here, we propose to elucidate dielectrophoresis (DEP) as a powerful analytical technique for proteins, which has vast potential for a wide range of applications such as concentration, separation, fractionation, and purification. Since protein DEP behavior is not well understood, our detailed study provides novel information eventually optimizing this protein migration method for analytical applications. We fabricated insulator-based DEP devices with nm-sized constrictions, which create maximum gradient of $E^2$ as large as $10^{19}$ V$^2$/m$^3$. With these devices, DEP experiments were performed with immunoglobulin G (IgG) and $\beta$-galactosidase under various buffer conditions. We also utilized numerical simulations to aid our understanding of protein DEP.

First, we investigated the DEP behavior with the nanoconstriction devices and found positive DEP of IgG with 12 fold concentration enhancement, while negative DEP for $\beta$-galactosidase. These concentration distributions were in agreement with numerical simulations. Furthermore, conductivity dependent DEP behavior was explained by the variation of protein charge and electroosmotic flow influencing on protein DEP. Finally, we observed a unique voltage dependent protein concentration, suggesting the possibility of ion concentration polarization occurring at the nanoconstrictions. Our study thus provides valuable information to eventually improve novel protein DEP devices for separation, pre-concentration, and fractionation. Funding from NIH is gratefully acknowledged.
Separation Sciences: Bioanalytical and Pharmaceutical

Chromatographic Studies of Drug Interactions with Lipoproteins by High Performance Affinity Chromatography

High density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) are lipoproteins (LPs) shown to bind basic and neutral hydrophobic drugs in serum. This process impacts the distribution, delivery, metabolism, and excretion of such drugs and is important in determining drug activity, pharmacokinetics, and toxicity in the human body. Information about drug-lipoprotein interactions and the strength of these interactions can be useful in determining how these drugs are distributed after administration and to design personalized dosage regimens.

This research uses high performance affinity chromatography (HPAC) and microcolumns to study binding of the drug propranolol to immobilized LPs such as HDL, LDL, and VLDL. Through these studies, two types of interactions were found between the LPs and propranolol. The first interaction had a relatively high affinity and probably involved binding to apolipoproteins on the LP surface. This interaction was stereoselective for LDL, while the other LPs exhibited high affinity but no stereoselectivity. The second type of interaction had a lower affinity and probably involved partitioning of the drug into the non-polar core of lipoproteins.

These studies indicate that HPAC can be a useful tool in characterizing mixed-mode interactions, as can occur with complex particles like LPs. The affinity microcolumns containing immobilized LPs allowed these studies to be conducted using the same column for hundreds of experiments with short analysis times. The combined result of these advantages was the ability to quickly obtain precise data over a variety of drug concentrations. The results of these experiments indicate that similar microcolumns prepared with other LPs or biological membranes can be used in similar HPAC binding studies.

Acknowledgment - work supported by National Institute of Health grant R01 GM 044931 in facilities renovated with support under grant RR015468-01.

Keywords: Bioanalytical, HPLC Columns, Immobilization, Protein

Application Code: Bioanalytical

Methodology Code: Liquid Chromatography
We propose an efficient method development protocol by using a C18 column in water/acetonitrile and a phenyl column in water/methanol for the initial screening. Their orthogonal selectivity provides a quick way to select the right columns. Depending on how well the samples are separated in each column, further refinement in column selection and mobile phase conditions can be optimized.

If the initial separation shows promise with the C18 column, the mobile phase is adjusted or the column selection is narrowed down further to other hydrophobic columns such as C4, C8, or Cholester (cholesteryl functional group) column. The Cholesterol column exhibits similar hydrophobicity as a typical C18 but it has more shape selectivity. The improved separation on structurally similar compounds is most likely due to the cholesteryl functional group’s relatively rigid structure.

If the phenyl column shows better separation, further mobile phase refinement can be done or other columns with stronger \(-\)interactions such as NAP (naphthalene functional group) or PYE (pyrenylethyl functional group) can be tested. The two fused aromatic rings gave the NAP an enhanced \(-\)interaction. If more is needed for better separation, PYE with its 4-fused aromatic rings provide the strongest \(-\)interaction. The increased retention on unsaturated or conjugated small molecules could save in the analysis time and ease in method transfer effort.

An example of method development using this two-column screening system is shown. The separation of various catechins using a C18 column resulted in adequate separation but with remaining unresolved peaks. The same catechin mixture injected into the Cholesterol column was able to provide complete baseline separation for all peaks.

Another example is shown using an adrenal-cortical-hormone mixture that required stronger \(-\)interaction to be baseline resolved.
Vitamin D2 and D3 are very important fat soluble vitamins for human and animals. Their deficiency is not only contributed to osteoporosis and osteomalacia but also associated with a variety of other diseases such as cardiovascular disease and type 1 diabetes. At high doses, they may cause some harmful effects. Thus, it is necessary to accurately determine their concentration in foods and supplementary products. In this study, a robust HPLC method has been developed for simultaneous determination of vitamin D2 and D3 in pharmaceutical tablets and mushroom samples. The tablet samples were ground, extracted with 60% ethanol aqueous solution and hexane, and analyzed by a Dionex HPLC. A baseline separation of vitamin D2, D3 and sample matrices was achieved using a reverse phase C18 column within 23 min. A good linear calibration curve was obtained with the squares of correlation coefficients greater than 0.999. The developed method has been also successfully applied to determine vitamin D2 and D3 in vitamin fortified orange juice and mushroom samples.

Keywords: Bioanalytical, Food Science, HPLC, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Methods of improving protein A affinity chromatography, the most common method of capturing monoclonal antibodies from complex cell cultures, are of recent interest to alleviate the current production bottlenecks in downstream processing. This technique centers on the high specificity of protein A to the Fc region of IgG. Commercially available protein A sorbents suffer shortcomings including flow rate limitations, limited mass transfer rates, and high cost. To address the need for a cost-effective and robust protein A support, we present capillary-channeled polymer (C-CP) fibers modified with a protein A ligand. C-CP fibers are unique in shape due to eight capillary channels running axially down the entire fiber. When packed into a column, the fibers interdigitate to form open tubular-like channels allowing for high throughput and efficiency. Separations are performed at high linear velocity with low backpressures, while the nonporous polypropylene fiber surface yields favorable mass transfer rates, allowing for rapid and efficient adsorption of protein A. In initial proof-of-concept studies performed on C-CP fibers packed into 0.8mm i.d. capillary tubing with 0.01 mL column volume (CV), protein A adsorbed to the fiber surface (4.24 ± 0.44 µg/mg) and remained stable under a variety of common solvent conditions and throughout the course of experiments, capable of multiple capture and elut cycles. Capture and recovery of IgG was evaluated as a pure solution and in a competitive mixture, yielding an average binding stoichiometry of 2.9:1, while optimization of elution solvents yielded 80% recovery of IgG. With an optimized protocol, scale-up was evaluated on columns up to 1 mL CV to give insight into the shift from analytical to preparative scale separations.
Polysorbate 20 is a non-ionic surfactant commonly used in the formulation of protein pharmaceuticals to prevent the formation of aggregates and protect the protein from denaturation. It is very important to understand the status and stability of polysorbate 20 in protein formulations as the ester species in polysorbate 20 hydrolyze in aqueous solution over time and lose their surfactant activity. In this study, a novel approach using 2D-LC coupled with CAD and MS detection was first reported for the characterization and stability study of polysorbate 20 in the presence of protein formulation sample matrix. A mixed mode column (Waters Oasis Max) that has both anion exchange and reversed phase properties was used in the 1st dimension to remove the proteins in the formulation sample, and the esters in the polysorbate 20 was trapped and then analyzed by Acquity BEH C18 RP-UHPLC column in the 2nd dimension to further separate and characterize the ester subspecies. Another 2D-LC method using Dionex ProPac cation exchange column in the 1st dimension and the same RP-UHPLC in 2nd dimension were developed for the analysis of hydrolysis products of polysorbate 20. The stability samples of an antibody drug product were studied using these two 2D-LC-CAD-MS methods to separate, identify and quantify the multiple ester species in polysorbate 20, and also monitor the change of their corresponding hydrolysis products. This 2D-LC approach allows formulation scientists to study the state of polysorbate 20 in real time protein drug products and help understand the potential impact on the stability of the protein formulations.
Separation Sciences: Bioanalytical and Pharmaceutical

The Development of Unique HPLC and SFC Stationary Phases that Utilize Advanced Particle Technologies

Both Reversed-phase HPLC and SFC is widely used for separation of many chemical compounds. A majority of these separations are based on ODS type columns. However, retention and separation of various compounds have proven to be a challenge. Many of these types of compounds are unretained, poorly retained or unseparated on most conventional ODS reversed-phase columns, even when these ODS column are packed with highly efficient sub 2 particles. Fortunately, to deal with these types of analytes we can employ alternative modes of chromatography that use unique stationary phases containing polar groups, organic bases, fluorinated groups and other non-hydrocarbon functional groups. These columns can to be used in the traditional reverse phase mode as well as both SFC and “hydrophilic interaction chromatography” or HILIC. SFC uses supercritical CO2 along with an organic modifier such as methanol. HILIC chromatography uses mobile phases containing between 5 - 20 % water for the retention of polar compounds. These unique stationary phases are bonded to support materials that utilize advanced particle technologies. It will be demonstrated that the combination of unique stationary phases bonded to advanced particle technologies will improved separations and add flexibility to operating conditions.

Keywords: HPLC, HPLC Columns, SFC
Application Code: Pharmaceutical
Methodology Code: Separation Sciences
Bisphenol A (BPA) is a monomer found in the manufacture of many commercial products, such as beverage can liners and animal housing. Recent reports have shown that BPA is not as stable as previously thought. Upon exposure to BPA, laboratory studies have demonstrated striking biological implications including abnormalities in the development of the nervous and reproductive systems. Animal housing systems are often subject to light, heat, bacteria and animal byproducts throughout their use. It is unknown how each stress affects the leaching and breakdown of BPA from the plastic housing units.

This study is designed to further the interdisciplinary education of chemistry majors by applying analytical instrumental techniques to analyze biological samples. Chemistry students in an advanced analytical courses will develop suitable Gas Chromatography-Mass Spectrometry and High Pressure Liquid Chromatography methods to evaluate the degradation and transformation of bisphenol A when subjected to four different stresses and one non-stress condition. The stresses in this study include chemical, bacterial, thermal and photo degradation conditions.

Keywords: Biological Samples, Gas Chromatography/Mass Spectrometry, HPLC, Separation Sciences
Application Code: Bioanalytical
Methodology Code: Separation Sciences
Separation Sciences: Bioanalytical and Pharmaceutical

High Resolution Separation Media for High Throughput Monoclonal Antibody Analysis

Monoclonal antibodies (MAbs) represent the most important class of bio-therapeutic molecules that usually display complex micro-heterogeneity with several modifications such as oxidation, isomerization, deamidation, glycation, C-terminus lysine truncation and others. Due to these variations, quality control and stability assessment of MAbs can be very challenging tasks. The increasing number of new MAbs being developed in the pharmaceutical industry is driving a growing demand for improved high resolution stationary phases for characterization of MAbs.

Earlier, we introduced Thermo Fisher Scientific MAbPac™ strong cation-exchange phases based on 10 μm, 5 μm and 3 μm particle sizes in PEEK housings for high resolution and high-throughput variant MAb analysis. However, due to pressure limitations of the PEEK hardware the columns need to be operated at relatively low flow rates. There is a need in the industry for analytical columns that combine high resolution with high flow rate compatibility for faster throughput.

With the launch of a bioinert high pressure Bio-RSLC Ultimate HPLC system from Thermo Fisher Scientific with maximum pressure of 15000 psi, we have developed small particle columns that are suitable for high resolution MAb analysis. PEEK lined stainless steel bioinert column hardware that is suitable for operation up to 15000 psi is used to avoid metal interferences with analytes of interest. Both isocratic and gradient analysis are used to evaluate the efficiency, resolution and ruggedness of these new columns.

Keywords: Bioanalytical, Biopharmaceutical, Ion Exchange, Method Development
Application Code: Bioanalytical
Methodology Code: Separation Sciences
Segregation Sciences: Bioanalytical and Pharmaceutical

Abstract Title
Ultraviolet Radiation Enhances the Glycation of Human Serum Albumin: A Study Involving Quantification of Carboxymethyl Lysine Derivatives

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Abstract Text
The non-enzymatic glycation of human serum albumin (HSA) has been shown to lead to the formation of a polymorphic group of compounds referred to as Advanced Glycation Endproducts (AGEs). AGEs can form both in vitro and in vivo and in diseases such as diabetes are found to accumulate in patients’ tissues and body fluids. The in vivo accumulation of AGEs has been associated to many of the chronic conditions of diabetes including atherosclerosis, renal failure and cataract formation. Earlier studies reported that the exposure of collagen to ultraviolet C (UVC) promoted the formation of reactive oxygen species, chemicals known to accelerate the in vivo glycation of the protein. The objectives of this study were twofold: 1) to use a combination of analytical techniques for evaluating the effect of UVC on the glycation of human serum albumin by D-glucose, and 2) to quantify the levels of carboxymethyl lysine (CML) derivatives in glycated HSA by an enzyme-linked immunosorbent assay. Emphasis was placed on CML derivatives which been shown to serve as a biomarker of AGE and for assessing the extent of tissue damage by oxidative reactions. Fluorescence spectrometry, HPLC and MALDI-TOF experiments demonstrated the glycation of HSA with D-glucose and confirmed the enhanced effects of radiation on the glycation of the protein under physiological conditions of temperature and pH. Increases in HSA’s CML levels established the formation of AGEs and allowed for establishing changes in the protein’s chemistry.

Keywords: HPLC, Immunoassay, Mass Spectrometry, UV-VIS Absorbance/Luminescence

Application Code: Bioanalytical

Methodology Code: Liquid Chromatography
Monoclonal antibodies (mAbs) are multidomain proteins that are extensively used as a research tool in molecular biology and to develop them as therapeutics in medicine. In many cases, antibodies are engineered to contain surface cysteines for the site-specific conjugation of payloads in antibody drug conjugates. Half-mAb containing intact antigen binding site is getting attention in nano-bioengineering such as in the development of ultrasmall diagnostic nano probes. Half Antibody Fragments are being used to improve biosensor sensitivity without loss of selectivity. Half antibodies are useful as bio ligands in other type of biosensors too. Half-mAbs can be formed through the selective reduction of the disulfide bonds present in the hinge-region of the monoclonal antibody. The accessibility of these disulfide bonds make them susceptible to reduction by mild reducing agents such as 2-MEA. With the focus on developing highly focused therapeutics in an effort to avoid negative side effects commonly associated with traditional drugs, half-mAbs are being heavily studied due to their ability for drug conjugation, antibody immobilization, or enzyme labeling, all possible through the exposed sulfhydryl group intrinsic to the half-mAb structure. In light of the growing interest in half-mAbs, here we report the use of a silica-based 7.8 mm ID x 30 cm; 4 µm particle size and 25 nm pore size analytical chromatography column for the separation of intact monomer of mAb from the half mAb (75 kDa) or half mAb equivalents such as conalbumin (75 kDa) and Bovine Transferrin (76 kDa). We also used Trypsin 21 kDa as IgG light chain equivalent in this study. TSKgel SuperSW mAb HR analytical size exclusion chromatography column, packed using unique pore-controlled technology results into a shallow calibration curve in the region typical to monoclonal antibodies providing better separation with high resolution.
Miniaturized electrochemical sensors (potentiometric and amperometric) for ions, gases, and nutrients/metabolites (e.g., glucose, lactate, creatinine) have revolutionized the practice of critical care and emergency medicine by providing simple tools to measure an array of physiologically important species in small volumes of undiluted whole blood at a patient’s bedside. However, additional situations remain in which electrochemical sensors or other electrochemical devices could be used to enhance the quality of medical care or solve important biomedical problems (e.g., catheter induced infections). This presentation will focus on progress in three areas; 1) the use of miniature enzyme-based electrochemical sensors to detect glucose in tiny volumes of tear fluid, as a potential means to monitor blood glucose levels indirectly via a non-invasive method; 2) the use of novel nitric oxide (NO) release polymers to enhance the biocompatibility and analytical performance of implantable chemical sensors, including intravenous glucose sensing catheters; and 3) the development of a new generation of intravascular and urinary catheters that employ electrochemically modulated generation/release of NO from a reservoir of inorganic nitrite ions to prevent microbial biofilm formation and concomitant infections commonly associated with use of such catheters in hospitals. In this last area, it will be shown that electrochemical production of NO within a lumen of a multi-lumen catheter, even for relatively short periods of time each day (3 h) at very low NO fluxes, can dramatically reduce biofilm formation and the number of living bacteria adhered to catheter surfaces over a 7-d period.
Over the last two decades, it has become increasingly accepted that the neurovascular unit—composed of neuronal, glial and vascular elements—forms the basis of a central autonomic nervous system. While its functions are diverse, the neurovascular unit serves to support brain function by matching nutrient-rich blood flow with the metabolic demands of regional activity. It is understood that the products of local neurotransmission trigger this response, known as functional hyperemia; however, much remains to be learned regarding the actions and mechanisms of the chemical messengers involved. This information is crucial to understanding disease pathologies that involve dysregulation of cerebral blood flow (CBF)—including migraine, cerebral ischemia, and many forms of dementia—as well as to interpreting data from brain imaging techniques such as blood oxygen level dependent (BOLD) fMRI. Carbon-fiber microelectrodes used with fast-scan cyclic voltammetry can be used to detect several neurotransmitters in the brain as a result of their electrooxidation. These microelectrodes can be used to detect brain molecular oxygen as a result of their electroreduction. This multianalyte detection approach allows mechanisms of the neurovascular unit to be probed deep within the brain. Our investigations have revealed the roles of norepinephrine, dopamine, histamine, and serotonin in this regulation.
Drug and chemical probe discovery frequently relies on high-throughput screening (HTS). Most HTS is done in multi-well plates with fluidic manipulation by robots and assay detection by fluorescence in a plate reader. While powerful, the volume of reagent used becomes a significant expense, costing $20-$50k/screening campaign. Also, it can be challenging to develop fluorescent readouts for some enzymes and protein-protein interactions. We have developed high-throughput sample manipulation techniques in droplet format that allows reduction of reagent volume down to nanoliters or 1000-fold less than multi-well plates. We have also developed methods of interfacing the droplets to mass spectrometry and chip electrophoresis that allow samples to be analyzed at 1 - 10 Hz. We are presently exploring using these methods for difficult protein-protein interaction targets (Hsp70) and enzymes (e.g., sirtuins).
Near infrared spectroscopy offers the potential to quantify selected analytes in a non-destructive manner. The concept is to pass a band of near infrared radiation through the sample of interest and determine the concentration of the targeted analyte by a multivariate analysis of the resulting spectrum. The ability to gain analytical information without chemical reactions or physical separations permits in situ analyses without regard for altering the sample or process under investigation. The strong absorption properties of water over the near infrared spectrum create a unique analytical challenge for analytical measurements of biological samples. Key analytical parameters impacted by water absorption include optical throughput, temperature sensitivity, and solvent displacement effects caused by dissolution of solutes. This presentation will focus on understanding the parameters that impact analytical measurements with near infrared spectroscopy of biological samples. In situ quantification of urea for hemodialysis, glucose in living tissue, and glycerol as well as methanol during protein expression in Pichia pastoris bioreactors will exemplify the capabilities of this technology.
Macrophage polarization has become a topic of interest to the biomaterials community since the M2c macrophage phenotype is believed to promote wound healing. Macrophages can exist in a continuum of phenotypes from the classically activated M1 state (wound promoting) to the alternatively activated M2 state (wound healing), with the M2c phenotype being considered the most significant for wound healing to implants. Much of this research has used in vitro models and a lack of translational work to the in vivo setting causes significant technical challenges. In this study, microdialysis sampling probes (500 micron o.d. and 10 mm length) are used as “sensor” mimics. Microdialysis sampling probes are implanted into the subcutaneous space of male Sprague-Dawley rats and infused with different agents (IL-10 and dexamethasone) reported to alter macrophage polarization to the M2c state. Histological and chemical (cytokine measurements) endpoints are used to compare treatments. In addition to these studies, fluorescent-folate-based tagging agents specific for macrophages are being developed with in vivo fluorescence imaging techniques to better track macrophages to the implant site. The combination of these studies will allow a better elucidation of the steps needed to provide appropriate wound healing and tissue integration to implanted sensors. We acknowledge NIH EB 014404 for funding.

Keywords: Bioanalytical, Biological Samples, Biomedical, Biosensors
Application Code: Bioanalytical
Methodology Code: Sensors
The first FT-IR spectrometers fell into three categories:

• Medium resolution slow-scanning far-infrared interferometers that used a rotating chopper to modulate the beam (pioneered by Gebbie’s group in England)
• High-resolution step-scanning near-infrared interferometers with phase modulation (developed by the Connes school in France)
• Rapid-scanning low-resolution mid- and near-infrared interferometers (developed at Block Engineering in the USA).

The latter concept proved to be the most versatile and is the basis of most modern FT-IR spectrometers. The earliest rapid-scanning interferometers could be held in the hand but only had a resolution of 16 cm⁻¹; higher resolution measurements only became possible when the mirror motion was made smoother through the use of air bearings and laser fringe referencing was introduced. Block Engineering spun off a company, Digilab, to develop and market a minicomputer-based laboratory instrument capable of 0.5 cm⁻¹ resolution. Minicomputers such as the DEC PDP-8 were available before 1969; however, they only had an 8-bit word which was too short for interferograms measured with a 14-bit analog-to-digital converter. The commercial introduction of a minicomputer with a 16-bit word, the Data General Nova, overcame this limitation. The final hurdle to overcome was the need for a fast, reasonably sensitive detector, as bolometers of the type used in grating spectrometers were not fast enough to measure interferograms with a high frequency of over 1 kHz; triglycine sulfate pyroelectric bolometers proved to be the solution. In this talk, the reasons for subsequent interferometer designs will be discussed and the current state-of-the-art summarized.

Keywords: FTIR, Infrared and Raman, Instrumentation, Vibrational Spectroscopy
Application Code: Other (Specify)
Methodology Code: Vibrational Spectroscopy
During a career spanning over fifty years and ranging from the halcyon days of atomic and nuclear physics, through the early days of laser development, the spooky days of cold war infrared science, and on to the rapid growth of FTIR spectroscopy and eventually spectroscopic process analysis, I've sometimes found myself on the periphery of interesting and significant developments and in the shadow of great ones who came before. My talk will view this era through a lens distorted by the random events that have determined the course of my own journey through science, technology, and business.
The instrument now known as an FTIR grew out of work done at Block Associates in the early 60s. I will try to give the listeners a feel for those early days by recounting the exploits of the participants. These were very colorful and distinct personalities with Myron Block as the most outrageous. The center of development shifted to California a decade later. The cast of characters changed but not the intensity. I will explore this phase as much as decency allows.

Keywords: FTIR, Instrumentation, Molecular Spectroscopy, Vibrational Spectroscopy
Application Code: General Interest
Methodology Code: Molecular Spectroscopy
### Abstract Text

The application of IR spectroscopy to industrial measurement has developed along with instrumental developments over time. My contributions to this effort have been intertwined with Mike Doyle's since the beginning of my career, and this presentation will trace out this interaction over several decades, and show how Mike's creativity has mutated and propagated over time through the instrumentation industry.

**Keywords:** Absorption, Chemometrics, FTIR, Instrumentation

**Application Code:** Process Analytical Chemistry

**Methodology Code:** Molecular Spectroscopy
Learning to Think Inside the Box: Spectroscopy and Chemometrics Come of Age Together

To successfully exploit innovative spectroscopic hardware, we had to learn the difference between fitting noise and a fitting application. As advances in vibrational spectroscopic instrumentation provided easier access to data of ever increasing quality, multivariate analytical techniques (chemometrics) to extract useful information from the data, reliably and in real time, became increasingly important. As techniques to extract useful information from spectroscopic data became more widespread, the stability and reproducibility of spectroscopic measurements became increasingly important. In this fashion, improvements in hardware motivated improvements in software which motivated further improvements in hardware, fostering a perpetual cycle of innovation that is making the deployment of industrial spectroscopic instrumentation more routine every day.

Keywords: Chemometrics, Data Analysis, Infrared and Raman, Molecular Spectroscopy
Application Code: Process Analytical Chemistry
Methodology Code: Molecular Spectroscopy
In this presentation we will discuss different aspects of teaching separation science concepts to students at smaller, primarily undergraduate institutions. This includes a wide variety of activities, ranging from lecture and laboratory components of traditional quantitative analysis courses and instrumental methods courses, independent study projects during the academic year, and research projects during winter break and summer sessions. To facilitate student learning about liquid chromatography, we have developed a web-based, interactive HPLC simulator for reversed-phase separations. This tool is quite sophisticated in that it allows students to immediately observe the consequences of changes in several operating parameters in both the isocratic and gradient elution modes: flow rate, particle size, column length, column temperature, mobile phase composition, and type of organic modifier. Chromatograms are generated based on pre-loaded input data from actual experimental retention measurements at different mobile phase compositions, temperatures, and with different modifier types. This tool is freely available at www.hplcsimulator.org, and the authors of this presentation and several collaborators have developed a series of homework assignments that demonstrate ways in which the simulator can be used in a variety of different courses. In this presentation we will share our observations of student learning gains as a result of using this tool, and summarize the ways we have used it in different courses. In situations where class (laboratory) time and instrument resources are limited, we believe this tool can significantly augment classroom discussion. However, when time and resources permit, hands-on experience is still the best way to develop an in-depth understanding of how separations and the associated instrumentation work.
Teaching analytical chemistry at San Diego State University, like at any institution, presents certain challenges. In this instance, with analytical chemistry classes of 60-80 students, the ability to engage each student in the material is one of the greatest challenges. To overcome this I have employed classroom polling systems to allow for pressure-free, anonymous, responses to questions from the students. This allows the students to engage with the material right away and gauge their understanding. The overall results also allow me to gain an understanding of their level of comprehension while still in the lecture, allowing me to adapt on the fly. I have also applied this tool to a graduate level separations course. Though the number of students are fewer (~20), the majority of the students are not analytical chemistry majors; presenting the challenge of accommodating diverse backgrounds, often lacking the focus on materials crucial to the separation sciences. By using the polling to get feedback from the class I ensure that I do not only hear the most confident/dominant voices, but can get feedback from all students.

Outside of the classroom, the internet has a significant role to play in the instruction of our students, whether we like it or not. Therefore it is in our interest to make use of this tool to ensure that our students are best served by it. I will present the two facets of internet instruction that I have been involved in: content creation and content curation. I have developed lab demo videos that have been viewed around the world, and I have been involved in the Analytical Sciences Digital Library, as a curator of the “best” materials available to assist instructors in teaching analytical chemistry and specifically separation sciences.

Keywords: Education, Separation Sciences, Teaching/Education
Application Code: Other (Specify)
Methodology Code: Education/Teaching
Chemical societies such as the American Chemical Society (ACS) provide accreditation criteria that guide the undergraduate curriculum, both in regard to content and delivery. For analytical chemistry, quantitative analysis and instrumental analysis textbooks yield additional guidance and uniformity for the curriculum. Finally undergraduate lectures on separation science are supplemented by laboratory experiments on techniques such as high performance liquid chromatography and gas chromatography.

At the graduate level the situation is vastly different. Professional societies and the chemical industry are silent as to what should be taught in a graduate course on separation science. Eminent chromatographers lament that “There is no good book on chromatography available at the graduate level”. And rarely, if ever, are there laboratory components in graduate courses to reinforce the lecture.

This presentation takes two approaches to address the question of what to teach and how to teach separation science at the graduate level. First, graduate programs from across North America have been surveyed with regard to content, class demographics, delivery mode, and resource materials used for separation science courses. Not surprisingly there is a wide diversity in both content and depth of discussion. This presentation will provide a meta-analysis of the topics covered, the emphases of discussion, and of the reference materials used. Second, specific innovative practices in teaching separation science will be highlighted. These include the use of chromatographic simulations, recorded lectures, integration of primary literature, and low-stakes and high-stakes writing assignments.

Keywords: Education, Gas Chromatography, HPLC, Separation Sciences
Application Code: Other (Specify)
Methodology Code: Separation Sciences
Modern technology affords us with many new ways to engage learners and to be innovative. Even so, traditional courses in Analytical Chemistry (and separation science, in particular) still rely heavily on textbooks and laboratory experiments to convey curricula. While chemistry as a whole will probably be the last hold-out in an increasingly on-line educational environment, due simply to safety considerations and equipment needs, we can explore new outlets and means to enrich the learning experience and provide continuing professional development for practitioners. An overview of efforts related to a) development of new laboratory curricula, b) on-line data handling and sharing, c) on-line instruction, d) use of social media, and e) good old fashioned textbook revision will be presented in this talk. The goal will be to reflect on the potential benefit of each component in the contemporary instruction of analytical chemistry.
I taught in the first ACS Short Course on Gas Chromatography in 1966. It was held in Pittsburgh and was a 2-day Lecture/Lab course with over 40 people registered. Dr. Steve dal Nogare of DuPont was the course director. I lectured and arranged for 4 simple GCs to be shipped in from Varian Associates, Walnut Creek, CA. I was Director of International Operations of Varian and had been a summer intern with Dr. dal Nogare in 1957 and a friend. I had introduced commercial GC Short Courses in Varian and thought highly of this teaching method. I joined Virginia Tech and part of my assignment was to bring ACS Short Courses to Va Tech. I introduced 41/2 day lecture/lab short courses on GC, HPLC, GC/MS and Sample Prep and taught them at VaTech until my retirement in 2003. Some one estimated I had taught over 10,000 students in short course over 40 years. I also taught the ACS short course on GC in Brazil and India. This lecture will summarize my many experiences and conclusions on this method of teaching.

Keywords: Chromatography, Teaching/Education
Application Code: Environmental
Methodology Code: Education/Teaching
Ultra-short (femtosecond) pulsed lasers offer compelling advantages for direct solid sample laser ablation (LA) chemical analysis with detection of elements and isotopes using ICP-MS (Inductively Coupled Plasma – Mass Spectrometry). The femtosecond laser reduces matrix effects due to a nominal heat-affected zone (no fractionation) and provides a nano meter-size particle aerosol that is ideal for transport, vaporization and ionization in the ICP. A lower energy and higher repetition rate from the laser produce the fine aerosol. Precision, sensitivity and accuracy of chemical analysis are improved compared to using longer pulse duration (nanosecond lasers). In addition, the low energy, high repetition rate femtosecond ablation process is ideal for rapid bulk analysis and mapping, both in 2D and 3D. This paper will present a brief overview of the fundamental differences between nanosecond and femtosecond laser ablation for chemical analysis and a summary of several applications including analysis of thin-film solar cells, battery materials and petroleum.
Advances in Mass Spectrometry Based on Ultrashort Pulse Laser Technology

Ultrafast Lasers Enable Non-Statistical Ion Activation and Sub-Cellular Atmospheric Pressure Chemical Imaging

Femtosecond lasers have traditionally been complex, expensive and unreliable, and hence incompatible with the requirements for analytical instrumentation. Advances in ultrashort pulse technology have greatly improved the reliability, user friendliness and even price of these sources. The time is approaching when ultrashort pulse lasers can form an integral part of analytical instruments, especially when these sources solve problems that cannot be addressed by traditional means. This talk will discuss pioneering research from my group on the use of femtosecond lasers in conjunction with mass spectrometry. I will present the use of phase and polarization shaped laser pulses for isomer identification and quantification. I will then discuss how femtosecond lasers solve the longstanding problem of post-translational analysis in proteomics, by causing non-statistical activation; i.e. breaking strong chemical bonds while leaving weak bonds intact. Finally, I will describe results on the use of femtosecond lasers to achieve sub-cellular atmospheric pressure chemical imaging.

Keywords: Chemical Ionization MS, Electrospray, Environmental/Biological Samples, Instrumentation
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
Advances in Mass Spectrometry Based on Ultrashort Pulse Laser Technology

High Pressure Femtosecond Laser Ionization Mass Spectrometry

Non-resonant strong-field ionization by intense focused femtosecond laser pulses (fs LI) is known to be universal, highly efficient and relatively "soft" in high vacuum environments. However, adoption of fs LI in mass spectrometry has been limited by the micrometer scale dimensions of the ionization volume which, coupled with low analyte density, provide poor sensitivity. We show that, by adapting an atmospheric pressure sampling mass spectrometer such as typically used for LC-MS, fs LI can be applied at atmospheric pressure and above. We refer to this as High Pressure Femtosecond Laser Ionization (HP fsLI). After characterizing the technique by studying Xe ionization, we show that HP fsLI retains the universality, high efficiency and softness of fs LI while achieving sensitivities that better electron impact ionization. In addition to direct ionization, conditions in the source can be adjusted to allow efficient laser seeded chemical ionization (CI) as an extension of the technique.

Direct application of the technique is anticipated in the analysis of gas streams from separation methods such as gas chromatography (GC) and cryogenic gas exchange. The gas throughput of an HP fsLI can be matched to the flow from a capillary GC allowing efficient sampling. We demonstrate the performance of HPfsLI used as the ionization technique in a GC/MS configuration. We find a tenfold improvement over electron impact ionization using our present system. By better matching flow rates and using higher repetition rate lasers we expect to be able to improve this performance by up to three orders of magnitude.

Keywords: Gas Chromatography/Mass Spectrometry, GC-MS, Laser, Mass Spectrometry
Application Code: General Interest
Methodology Code: Mass Spectrometry
The use of femtosecond laser vaporization as a release method for nonvolatile molecules was reported by this laboratory for a series of macrocyclic molecules (RCMS 2009, 23(19), 3151). In this experiment, an 800 nm pulse of duration 60 fs and intensity $10^{13}$ W cm$^{-2}$ transfers molecules into the gas phase for subsequent electrospray pickup and mass analysis. A femtosecond duration vaporization pulse allows universal delivery of sample into an electrospray source for a wide variety of condensed phase states ranging from solids, liquids, and tissues without the need to add an external matrix. The capability to detect pharmaceuticals, explosives, improvised explosive mixtures, lipids, peptides, and proteins has been demonstrated. The method has been shown to be quantitative for a number of polar and nonpolar molecules (JASMS 2011, 22(4) 762). This contribution will focus on the quantitative analysis of mixtures of proteins, where conventional electrospray fails due to charge competition and saturation effects (AC, in press). The mechanism of quantitative analysis will be discussed as well as applications in phenotyping (AC 2012, 84(14) 6225), protein structure determination (PNAS 2011, 108(30) 12217), and imaging mass spectrometry.

**Keywords:** Forensics, Laser Desorption, Mass Spectrometry, Protein

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
The first atomic view of strongly driven phase transitions (Siwick et al, Science 2003) illustrated the mechanism to control nucleation growth to nm scales (10 atoms/molecules). To take advantage of this new insight, a laser concept was developed based on a seeded Optical Parametric Amplifier and microchip laser technology to provide a compact robust source engineered to excite the OH stretch of water in biological tissue for use in laser surgery. It was discovered that this new mechanism ejects entire proteins into the gas phase intact, and most importantly the damage to surrounding tissue was negligible, with no scar tissue formation (Amini-Nik et al, PLoS 2010). This is the first method, by any means, capable of surgery without scar tissue formation. The long held promise of the laser for achieving the fundamental (cell) limit to surgery has now been realized. In the process, it was also discovered that entire proteins, even protein complexes, are ejected into the gas phase intact, with the whole process of vibrational excitation, coupling to translational motion, and ablation, occurring faster than even collisional exchange of the excited water with the proteins. This new laser ablation mechanism referred to as Desorption by Impulsive Vibrational Excitation (DIVE) provides a new means for in situ spatial mapping with mass spectroscopy in which preliminary results show very detailed molecular signatures of different tissue types. An imaging mass spectrometer is being designed, based on lessons from high brightness electron source development, that should be capable of near unit ion and detection efficiency, to provide significant gains in sensitivity. The basic theory for the laser ablation process, as well as applications for mass spectroscopy as feedback in laser surgery, and towards fundamental limits in spatial mapping and biodiagnostics, will be discussed.

Keywords: Bioanalytical, Biological Samples, Biomedical, Laser
Application Code: High-Throughput Chemical Analysis
Methodology Code: Mass Spectrometry
Metabolic phenotypes can provide a window onto dynamic biochemical responses to physiological and pathological stimuli. Metabolic profiling strategies for analyzing biosamples, encompassing high-resolution spectroscopic methods (NMR spectroscopy, LC-MS, GC-MS etc) in combination with multivariate statistical modelling tools, have been shown to be well-suited to generating metabolic signatures reflecting gene-environment interactions. Spectroscopic analysis has been applied across a wide range of studies with the aim of characterizing classes of disease, different physiological states or response to particular therapies and the natural extension is to derive predictive models for metabolic response from a baseline profile. A range of chemometric and bioinformatics tools has been developed to accommodate complex biological spectroscopic data ranging from simple multivariate linear projection methods such as principal components analysis to Bayesian probabilistic modelling and other nonlinear methods. In addition to data modelling tools, a suite of spectral preprocessing algorithms has been generated to align, normalize and scale data. Specific challenges to analyzing spectral data are the difficulty in automated annotation of spectral peaks, the extreme inter-individual variation that can arise in human studies due to genetic and environmental exposures and confounding factors such as drug interventions in disease studies that result in both spectral contamination and in pharmacological responses that make identification of pathological mechanisms difficult to extract. Methods for characterizing the metabolic consequences of biological processes will be discussed with particular emphasis on accommodating extraneous variation and optimizing biomarker recovery. Additionally a framework for predicting response to interventions at the individual level will be presented and examples drawn from a selection of laboratory and clinical studies.
### Abstract Title
Isotopic Ratio Outlier Analysis (IROA) and Imaging Mass Spectrometry in Metabolomics

### Abstract Text
Metabolomics is a fast growing field in mass spectrometry, thus it is hard to state what would be the traditional approach to globally identifying all small molecules in a given system. However, most work involves the use of both high resolution chromatography (UHPLC) and high resolution mass spectrometry in order to separate out the individual small molecules from the noise of a biological sample. Imaging mass spectrometry involves the direct probing of tissue sections for chemical signatures from given areas (i.e. spatial separation) of a tissue or between two sets of tissues. This offers tremendous potential to rapidly probe metabolomic signatures with little to no sample preparation, thus providing a unique signature of a specific condition. IROA involves the use of stable isotopes to probe chemical processes based on direct metabolism of the stable isotopes. This approach helps to reduce artifact detection and more rapidly identify those species that are changing. This talk will summarize the two technologies and look at how they will impact the field of metabolomics in the near future.

### Keywords
- Biomedical
- Imaging
- Mass Spectrometry
- Metabolomics

### Application Code
Biomedical

### Methodology Code
Mass Spectrometry
Oligosaccharides are composed of 3-20 monosaccharide units that are found in biological fluids as free oligosaccharides or as glyconjugates in proteins and lipids. While sequence defines nucleotides and peptides, oligosaccharides are complicated by numerous linkages, branching, and stereomeric residues. For this reason, the analyses of oligosaccharides or glycans have not progressed as rapidly as it has in genomic and proteomic. Nonetheless, understanding the roles of oligosaccharides depends on the ability to quantitate hundreds of structures simultaneously. Research in our laboratory has focused on the rapid quantitation of oligosaccharides so that analysis can be performed on hundreds of samples in different matrices. Methods are developed to rapidly identify structures based on a library with three key parameters: accurate mass, chromatographic retention times, and tandem mass spectrometry. Structures in the library were first determined by tandem mass spectrometry and the strategic use of exoglycosidases. A library of over 100 structures and over 200 entries has been constructed. For high sensitivity, nanoflow liquid chromatography is used to isolate and yield retention indices. The majority of the structures can be identified strictly based on accurate masses and retention times. However, the addition of tandem mass spectra provides unambiguous determination of the compounds identity. Quantitation is performed based on chromatographic peak area, stable isotope labeling and multiple reaction monitoring.
Despite major advances in the fields of microbial pathogenesis and medicinal chemistry, only 3 new chemical classes of antibiotics have been approved in the past 20 years. The causes of this shortfall are multifactorial. However, growing evidence has highlighted key dissociations between our understanding of the biology of these pathogens and the current drug development toolbox. Here, we highlight the merging potential of metabolomics technologies to bridge these gaps and reinvigorate the anti-infectives pipeline.
Identifying the ‘Dark Matter’ in GC/MS and LC/MS Experiments

Despite the availability of comprehensive electron ionization libraries for GC/MS, a substantial fraction of the signal acquired in many of these experiments arises from unidentified compounds. With smaller libraries for LC/MS and the common occurrence of multiple ions for single components, the situation is even worse in these experiments. Even in ‘bottom-up’ proteomics experiments, where the peptide products can often be identified by matching in-silico spectra, a substantial fraction of components are not identified. Further, in all of these cases the analyst is provided little information concerning the magnitude, or even the presence of this unidentified signal (‘dark matter’). In this presentation we describe methods we have developed for extracting spectra for unidentified components from these systems, enabling the labeling and annotation of ‘recurrent spectra’ in other experiments of related mixtures. At the least, these provide useful information for quality control – at most they can identify promising biomarkers. These methods employ spectral clustering and refinement methods to derive ‘consensus spectra’ for unidentified components and a special adaptation of AMDIS for deriving components from GC/MS data files. Recurrent spectral libraries derived for specific matrices, combined with comprehensive libraries of reference spectra for known components promise to provide a far more detailed determination of all of the chemical constituents in a wide range of biological, environmental or industrial mixtures.

Keywords: Bioinformatics, Gas Chromatography/Mass Spectrometry, GC-MS, Metabolomics, Metabonomics
Application Code: Bioanalytical
Methodology Code: Gas Chromatography/Mass Spectrometry
One of the most important processes in metabolomics is compound identification. The spectrum matching-based method is the widely used approach for compound identification in GC-MS based metabolomics, where the mass spectral similarity between an experimental mass spectrum and each spectrum in a reference library is calculated. While various similarity measures have been developed to improve the overall accuracy of compound identification, no attention has been, in general, paid to reducing the false discovery rate. We first investigated the effect of the isomers in compound identification and found that the isomers are one of the major bottlenecks for high accuracy compound identification because their mass spectra are often very similar to each other and therefore, indistinguishable in terms of spectral similarity score. For this reason, we focused on the first and the second highest similarity scores of a query and developed an approach for controlling false identification rate using the distribution of their difference. We further proposed a model-based approach to achieving a desired true positive rate. The developed method was applied to the NIST mass spectral library and its performance was compared with the conventional approach that uses only the maximum spectral similarity score. The results show that the developed method achieves a significantly higher F1 score and positive predictive value than those of the conventional approach.

**Keywords:** GC-MS, Identification, Metabolomics, Metabonomics

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

**Methodology Code:** Chemometrics
ADAP-GC 2.0 has been developed to deconvolute coeluting metabolites that frequently exist in real biological samples of metabolomics studies. Deconvolution is based on a chromatographic model peak approach that combines five metrics of peak qualities for constructing/ selecting model peak features. Prior to deconvolution, ADAP- GC 2.0 takes raw mass spectral data as input, extracts ion chromatograms for all the observed masses, and detects chromatographic peak features. After deconvolution, it aligns components across samples and exports the qualitative and quantitative information of all of the observed components. Centered on the deconvolution, the entire data analysis workflow is fully automated. ADAP-GC 2.0 has been tested using three different types of samples. The testing results demonstrate significant improvements of ADAP-GC 2.0, compared to the previous ADAP 1.0, to identify and quantify metabolites from gas chromatography/time-of-flight mass spectrometry (GC/TOF-MS) data in untargeted metabolomics studies.
Bioinformatics: Metabolite Identification and Quantification

Strategies to Improve High-Throughput Identification in Untargeted Metabolomics

Mass spectrometry-based metabolomics relies on MS/MS data for structural characterization of metabolites, however, MS/MS data acquired during untargeted profiling commonly contain artifacts that prevent metabolite identification. Here we introduce an approach to experimentally remove MS/MS artifacts from untargeted metabolomic data and thereby reduce the frequency of false-negative assignments. Our strategy relies on evaluating MS/MS fragment intensities as a function of retention time, precursor mass targeted for MS/MS, and sample class. We describe an untargeted metabolomic workflow consistent with this approach that uses less stringent isolation settings, thus offering the additional benefit of improved sensitivity at the expense of specificity. The value of our approach is highlighted by showing structurally characterized metabolites in brain tissue that would have otherwise gone un-identified.

Biological Samples, Data Analysis, Liquid Chromatography/Mass Spectroscopy, Tandem Mass Spec

Genomics, Proteomics and Other 'Omsics

Mass Spectrometry
Due to the high complexity of metabolome, the comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-TOF MS) is considered as a powerful analytical platform for metabolomics study. However, the applications of GC×GC-TOF MS in metabolomics are not popular due to the lack of bioinformatics system for data analysis. We developed a computational platform entitled MetPP for analysis of metabolomics data acquired on a GC×GC-TOF MS system. MetPP can process peak filtering and merging, retention index matching, peak list alignment, normalization, statistical significance tests, and pattern recognition, using the peak lists deconvoluted from the instrument data as its input. The performance of MetPP software was tested with two sets of experimental data acquired in a spike-in experiment and a biomarker discovery experiment, respectively. MetPP not only correctly aligned the spiked-in metabolite standards from the experimental data, but also correctly recognized their concentration difference between sample groups. For analysis of the biomarker discovery data, a total of 15 metabolites were recognized with significant concentration difference between the sample groups and these results agree with the literature results of histological analysis, demonstrating the effectiveness of applying MetPP software for disease biomarker discovery.
Endogenous opioid function has been implicated in specific aspects of both neuropathic pain and reward in models of addiction. In order to understand the precise molecular mechanisms involved in the neural circuits that mediate pain and reward, an analytical method is required for real-time detection of endogenous opioid release from cells in living tissue. Electrochemical approaches coupled with carbon-fiber microelectrodes have sufficient speed, sensitivity, and spatial resolution for detecting the vesicular release of catecholamines from single cells, as well as for the detection of discrete catecholamine fluctuations in vivo. However, many challenges exist for applying an electrochemical approach to the detection of neuropeptides. We have developed and characterized a novel voltammetric waveform for the detection of tyrosine-containing neuropeptide molecules, such as met-enkephalin (mENK), using fast-scan cyclic voltammetry at carbon-fiber microelectrodes. We have established that the main contributor to the electrochemical signal is tyrosine, and that conventional waveforms provide poor peak resolution and allow fouling of the electrode surface. With our analyte-specific waveform we have selectively distinguished mENK from common endogenous interferents, such as dopamine and pH shifts, and have detected mENK release in brain tissue and from cells in the adrenal medulla. This work provides a foundation for real-time measurements of endogenous mENK, and is broadly applicable to the voltammetric detection of a variety of tyrosine-containing neuropeptides.
Electrochemical detection is an excellent method for detection of neurotransmitter release during exocytosis events because it is fast, sensitive and the surface of the electrode can be held close proximity to the surface of a single cell. We have used the two-enzyme system consisting of acetylcholine esterase and choline oxidase to construct an enzyme-based electrochemical sensor for detection of the non-electroactive neurotransmitter acetylcholine. The catalytic reaction of these two enzymes can convert the non-electroactive acetylcholine molecules to an electroactive secondary product, such as hydrogen peroxide. In this work we used a carbon fiber microelectrode functionalized with gold nanoparticle to the surface, to increase the effective surface area and to retain the activity and secondary structure of the enzymes bound to the highly curved surface area. This work was first started by performing a careful characterization of the gold nanoparticle enzyme conjugates using these two enzymes to determine the enzyme coverage on the nanoparticles and to determine the enzyme activity. This was used to find the optimal conditions for co-immobilization of the two enzymes to the electrode surface and to maintain the highest retained enzyme activity. The sensor response was tested using an artificial cell model for exocytosis where single vesicle release events were recorded and with a time-response of half the rate of single vesicle release events with electro active molecules, but still fast enough to resolve single vesicle release events.

Keywords: Bioanalytical, Biosensors, Neurochemistry

Application Code: Bioanalytical

Methodology Code: Electrochemistry
We have developed a new electrochemical imaging technique, fluorescence-enabled electrochemical microscopy (FEEM), for studying highly dynamic heterogeneous redox processes. A central idea of FEEM is the electrical coupling between a conventional faradaic process and a fluorogenic process at a closed bipolar electrode. This allows us to use highly sensitive fluorescence to monitor almost any conventional electrochemical processes, e.g., oxidation of dopamine, at numerous parallel ultramicroelectrodes (UMEs). In this talk, I will describe our new progress on using well-defined electrochemical arrays to image highly dynamic redox processes. I will demonstrate how nanoelectrode arrays enable us to approach nanoscale spatial resolution. This work has been supported by NIH and NSF.
We have fabricated and characterized microwell-based individually addressable microelectrode arrays (MEAs) and applied them to spatially and temporally resolved detection of neurotransmitter release across a single pheochromocytoma (PC12) cells. The microwell-based MEAs consist of sixteen 4-µm-width square ultra-microelectrodes, or twenty-five 3-µm-width square ultra-microelectrodes, or thirty-six 2-µm-width square ultra-microelectrodes. These ultra-microelectrodes in each MEA are tightly defined in a 30×30 µm square area, which is further encased inside a 40×40 µm SU-8 microwell. Microwell-based MEAs were fabricated on glass substrates by photolithography, thin film deposition, reactive ion etching and other semiconductor fabrication techniques. These electrode arrays have excellent stability and reproducibility as demonstrated by use of cyclic voltammetry and performed recording of spatially resolved exocytosis across cells with multiple ultra-microelectrodes. Effective targeting and culture of a single cell is achieved by combining cell-sized microwell trapping and cell-picking micropipette techniques. The surface of microelectrodes in the MEA was coated with collagen IV to promote cell adhesion and further single cell culture. Experience shows that good adhesion between cell membrane and electrode surface is very important to measure the exocytosis from the basal side. Amperometric results show that the subcellular heterogeneity in single-cell exocytosis can be electrochemically detected with these microwell-based MEAs. These MEAs are suitable for detecting fast chemical events at single cells, as well as for developing multifunctional electrochemical sensors. Furthermore, we have been working with collaborators to develop biosensors on electrode arrays. One novel biosensor is that for ATP, a molecule that is also released during exocytosis. The sensor design promises to be fast with millisecond response time and sensitive, at micromolar levels.
**Session Title:** Global Challenges in Food Safety  
**Abstract Title:** The Impact of Globalization of the Food Supply on the Analytical Laboratory

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|----------------|----------------|

**Abstract Text**

The U.S. Food and Drug Administration’s (FDA) Office of Foods and Veterinary Medicine was created to lead a functionally unified Foods Program and enhance the Agency’s ability to meet today’s great challenges in food and feed safety, nutrition, and other critical areas. The FDA Foods Program includes the Center for Food Safety and Applied Nutrition (CFSAN) and the Center for Veterinary Medicine (CVM), working closely with the Office of Regulatory Affairs (ORA).

CFSAN’s role in food analysis is to assure that the nation’s food supply is safe, sanitary, wholesome, and honestly labeled. This has been complicated over the last several decades by a tremendous rise in imported foods. In 2010 alone, FDA estimates that more than 20 million import lines of regulated products, from more than 150 different countries, arrived at 300 U.S. ports of entry—more than a three-fold increase from only a decade ago. The vast number of these imports provide for a safe, year-round supply of fresh foods. Unfortunately, the global supply chain has also led to the distribution of unsafe products caused by economic adulteration and intentional fraud. In order to defend the food supply from these unsafe products, a variety of new, innovative approaches to foods analysis are required. One of the main research thrusts is improving regulatory methods, which includes both method development and rigorous validation. Along with providing methods for planned sample analysis to inform the Center’s regulatory decisions, scientists must also be working on methods to respond to emerging issues (e.g. nanotechnology) and emergencies (e.g. Melamine). An overview of the Agency’s role in foods research relating to analytical chemistry and case studies will be presented.

**Keywords:** Agricultural, Bioinformatics, Food Science, High Throughput Chemical Analysis

**Application Code:** Food Science

**Methodology Code:** Chemical Methods
Global Challenges in Food Safety

Challenges in Monitoring Chemical Contaminants in Food

According to the World Trade Organization, global trade of food is currently a $1.4 trillion market, which is nearly 8% of all international trade. All countries have established laws or follow international guidelines that limit permissible levels of chemical residues and contaminants in food. Analytical monitoring is often conducted by contract laboratories, food industry, and regulatory agencies to check that the food is not adulterated and meets quality standards. The amount of testing of foods has increased greatly due to growing food trade, expanding exports from developing nations, and previous food scares due to chemical contaminants, such as the melamine crisis in 2007-2008. One analytical challenge is the higher priority placed on non-targeted screening of as many potential contaminants as possible in the food. Another major challenge in monitoring programs due to the perishability and economics of food is that sample throughput must be maximized at minimum expense. Also, growing importance of exceptionally complicated food matrices such as tea, coffee, spices, and nutraceuticals constitute a very difficult challenge in sample preparation and analysis. State-of-the-art sample preparation, chromatography, and mass spectrometry are meeting some of the challenges described above, and current pitfalls/limitations will be discussed in this presentation.

Keywords: Agricultural, Chromatography, Mass Spectrometry, Sample Preparation

Application Code: Regulatory

Methodology Code: Chemical Methods
Chemical contamination of food can originate from a variety of sources throughout the food supply chain. The term food contaminant covers a wide range of compounds and their presence may be of concern due to implications for food safety, regulatory compliance, or quality of products and consumer perception. The levels of contaminants observed can vary greatly and analytical methodology must be fit for purpose to enable accurate detection down to required levels, or be sufficient to demonstrate absence as low as reasonably practical for some banned substances. If the organoleptic properties of a food are not as expected, it can result in a lack of consumer confidence and a perception of poor quality, leading to customer complaints, brand damage and potentially a costly product recall. Therefore, the identification of compounds causing taints and off-flavours in food is a major concern to the food industry, even when the compounds are not present at a level to cause a safety issue.

The determination of taints and off-flavours in food can be particularly challenging due to the need for sensitive methodology and the commercial pressure for rapid results. Often the analyte is unknown and the compound(s) responsible for the taint may be present at extremely low levels (sub ppb). Expertise in data interpretation is crucial and the link of sensory, analytical data and background information about each case must be assessed to complete the investigation and prevent future occurrence.

This presentation will cover some of the analytical techniques employed for determination of of unknown contaminants in food and also discuss the implications of miniaturization, and developments in instrumentation and software.

Keywords: Analysis, Contamination, SPME, Ultratrace Analysis
Application Code: Food Science
Methodology Code: Separation Sciences
The FDA's Forensic Chemistry Center (FCC) provides laboratory support and problem solving capabilities in emergency response to events of chemical adulteration and contamination of foods. Contamination may be intentional as in cases of product tampering, economic adulteration, and terrorism. Unintentional contamination, such as in cases of environmental or process induced contamination, have also required emergency response. Typically, a broad based approach is used to initially characterize the contamination using a variety of analytical tools and when possible provide information about the source of the contamination. Once a problem is characterized, it is often necessary to focus the required analyses and to transfer methodology to additional laboratories. A number of factors must be taken into account when responding to these emergencies including, for example, choice of appropriate methodology, validation requirements, instrument availability, method complexity, and analysis time. Several case studies encountered over the past few years will be presented.

Keywords: Chemical, Food Science, Forensic Chemistry
Application Code: General Interest
Methodology Code: Other (Specify)
The increasing need for carbon free energy has focused renewed attention on solar energy conversion. Direct photoelectrolysis of water has the advantage of converting solar energy directly to hydrogen, an ideal non-carbon and nonpolluting energy carrier, by replacing both a photovoltaic array and an electrolysis unit with one potentially inexpensive device. Unfortunately no materials are currently known with the required properties to efficiently photoelectrolyze water that are stable under illumination in electrolytes for many years. Nanostructured semiconducting metal oxides could potentially fulfill these requirements, making them the most promising materials for solar water photoelectrolysis, however no oxide semiconductor has yet been discovered with all the required properties. We have developed a simple, high-throughput combinatorial approach to prepare and screen many complex oxides for water photoelectrolysis activity. The approach uses ink jet printing of overlapping patterns of soluble metal oxide precursors onto conductive glass substrates. Subsequent pyrolysis produces metal oxide phases that are screened for photoelectrolysis activity by measuring photocurrents produced by scanning a laser over the printed patterns in aqueous electrolytes. Several promising and unexpected compositions have been identified. In addition, due to the millions of possible combinations to be printed and screened, we have developed a distributed research project that uses simple and inexpensive printing and screening devices, such as Lego Mindstorm® kits, to enlist many undergraduate and high school student researchers into the search for the “Holy Grail” of materials. The Solar Hydrogen Activity research Kit or SHArK project has now expanded to over sixty sites.
Understanding interfacial charge transfer events at the nanometer scale is essential for providing guidelines for optimizing performance of various electrochemical devices. Combined methods of traditional electrochemistry and high spectral/spatial resolution optical spectroscopy provide tools to study these local electrochemical processes. We used electrogenerated chemiluminescence (ECL), dark field light scattering, and single molecule fluorescence of redox species as powerful tools to measure the interfacial charge transfer events at nanostructured electrodes, and redox activities of single molecule and single nanoparticles. Our results also show that these optical spectroscopy methods enable the exploration of electrochemical events with good temporal and spatial resolution without obstruction from the ensemble averaging inherent in conventional electrochemical methods.

Keywords:     Electrochemistry, Electrodes, Materials Science, Nanotechnology
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Electrochemistry
Apart from the enhanced activity, reaction selectivity plays a very important role in practical (electro)catalysis. In this talk, I will demonstrate how the selectivity of electrocatalysts may be steered by catalyst surface structure, catalytic promoters, and solution composition including pH. Examples include CO₂ reduction, nitrate reduction, and glycerol oxidation. The origin of the catalytic enhancement is studied by a variety of electrochemical techniques coupled with first-principles density functional theory calculations. The online detection of products during cyclic voltammetry requires the development of analytical setups such as online electrochemical mass spectrometry, and online fraction sampling coupled with HPLC and IC.

**Abstract Text**

**Keywords:** Electrochemistry, Electrode Surfaces, HPLC Detection, Ion Chromatography

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

**Methodology Code:** Electrochemistry
The design of better electrodes for secondary battery technologies, i.e. those based on intercalation of alkaline and alkaline-earth metal ions, require a fundamental understanding of their driving processes and the relationships between them. Crucial knowledge regarding the spatial and kinetic heterogeneity of ion intercalation processes in battery electrodes at different scales, from single particles to composites, need to be generated. Furthermore, how these ionic processes relate to localized electron transfer processes (e.g. conductivity and reactivity, catalyzed electrolyte decomposition, the role of defects) needs yet to be established in a versatile manner through the use of scalable, spatially resolved and chemically-specific probes.

Here we present our approach to understanding battery electrode interfaces. Our technique is based on the use of novel amperometric probes capable of quantitatively detecting ionic fluxes. These are used in conjunction with nanoelectrodes capable of measuring local electron transfer rates and catalytic activity. These probes are integrated into the Scanning Electrochemical Microscope, which allows us to generate reactivity maps of model engineered interfaces in battery electrolytes under stringent inert atmosphere conditions. These probes are multi-scale in that they use similar operation principles to generate information for processes occurring from the millimetric to the nanometric domains. In this presentation, we introduce the first steps towards the imaging and measuring capabilities of these probes on a model carbon material. The correlation between ionic and electrochemically active sites as a function of electrode operation is discussed.

Keywords: Electrochemistry, Electrode Surfaces, Energy, Ion Selective Electrodes
Application Code: Materials Science
Methodology Code: Electrochemistry
The INLIGHT strategy for the sample preparation, data analysis, and relative quantification of N-linked glycans will be presented. Glycans are derivatized with either natural (L) or stable-isotope labeled (H) hydrazide reagents and analyzed using reversed phase liquid chromatography coupled online to a Q Exactive mass spectrometer. We demonstrate that the INLIGHT approach can quantify species over four orders of magnitude in ion abundance. The INLIGHT strategy is further demonstrated in pooled human plasma, where it is shown that the post-acquisition normalization is more effective than using a single spiked-in internal standard. We have applied the INLIGHT strategy to a unique bio-respository of human Ovarian Cancer (OVC) samples and also to longitudinal samples from the domestic hen model of OVC. This work is supported by IMAT, a program in the National Cancer Institute.

Keywords: HPLC, Mass Spectrometry
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Mass Spectrometry
The biological activity and stability of proteins is influenced by their glycosylation. Many biopharmaceuticals are glycoproteins, and monitoring as well as controlling their glycosylation is of key importance for ensuring their quality, resulting in a need for fast, sensitive and robust methods for protein glycosylation analysis. Here, various methods for glycosylation analysis will be presented including UPLC with fluorescence detection, capillary gel electrophoresis with laser-induced fluorescence detection (CGE-LIF), as well as LC-MS and MALDI-TOF-MS of glycans and glycopeptides. The methods will be compared with regard to speed, robustness, throughput, and costs. Examples will be given of high-throughput glycosylation analysis for the detection of clinical biomarkers of autoimmune diseases and cancer.

Keywords: Biopharmaceutical, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry, Proteomics

Application Code: Bioanalytical

Methodology Code: Mass Spectrometry
Despite the rapid growth in innovation related to mass MS-based glycomics, accurate quantitative analysis of glycans still represents a formidable challenge in this area. Several strategies for glycan quantitation based on stable isotope reagents have been introduced in the past and applied to a limited number of samples for proof-of-concept demonstration only. Reducing end (aldehyde)-reactive Tandem Mass Tag (TMT) reagents have a potential of filling the gap and addressing the need for accurate relative quantitation of glycans. We have already demonstrated the utility of these reagents for quantitative analysis of native and permethylated N-glycans using MALDI and ESI-based MS instruments. In this work, we expand the scope of our study by further investigating their use on ESI-MS ion-trap and hybrid orbitrap-based instruments coupled with online LC separation. Tryptic digests of several standard glycoproteins were treated with PNGase F glycosidase to release N-glycans. Following reversed-phase extraction/desalting steps, the glycans were subjected to a reaction with aldehyde-reactive alkoxyamino TMT reagents. Native labeled glycans were analysed using Velos Pro dual-pressure linear ion trap and Orbitrap Fusion Tribrid (Thermo Scientific) instrument to assess MS/MS fragmentation patterns of glycans at different charge states with different metal adducts in different fragmentation modes.
Quantitative analysis of the carbohydrate moiety of glycoprotein biopharmaceuticals is of high importance, especially when glycosylation changes can impact the biological effect of the drug. In case of monoclonal antibody therapeutics, sialylation (NANA vs NGNA), altered galactosylation (the presence of 1,3-Gal residues) and the extent of core fucosylation may significantly influence function and immunogenicity, thus should be closely monitored during clone selection, product development and lot release. Comprehensive analysis of protein glycosylation represents one of the most challenging bioanalytical tasks as glycans are very complex molecules with their chemical natures ranging from neutral, linear polymer structures to branched, highly polar or even charged ones. The lack of chromophore / fluorophore and, in many instances easily ionizable groups usually require derivatization before their analysis by high performance bioanalytical techniques, such as liquid chromatography, electrophoresis and mass spectrometry. Full structural elucidation of glycans may also utilize consecutive enzymatic digestions by sugar and/or linkage specific exoglycosidases, followed by LC or capillary electrophoresis analysis of the digests. Hyphenation with offline weak anion exchange chromatography (WAX) fractionation is an additional powerful tool, especially when highly sialylated glycan structures are present. This presentation will cover the state of the art of liquid phase carbohydrate separation methods, mostly focusing on capillary electrophoresis and its combination with mass spectrometry. N-glycosylation analysis of this recently emerging class of very successful new generation drugs will be discussed with the main goal to demonstrate structural and functional equivalence of innovator and biosimilar products focusing on quantification of their core fucosylation degree as well as alpha-gal residue and N-glycolyneuraminic acid content.
Permethylation is currently a routine derivatization method in MS-based glycomics. This derivatization improves the ionization efficiency of glycans and allows separation on reversed-phase liquid chromatography (RPLC). Such quantitative glycomics approach has been effectively utilized to understand the biological attributes of glycans. However, RPLC separation of permethylated glycans at ambient temperatures is not efficient, partially because of the low mass transfer between stationary phase and mobile phase. This is limited by the intermolecular interactions among the methyl groups present on permethylated glycan branches. Performing the RPLC separation at high temperatures (45-75[^°]C) improved separation efficiency and selectivity. Better peak symmetry was observed at the elevated temperatures. For example, the peak asymmetry factor of Man9 glycan improved from 3.63 to 1.19 as the column temperature increased from ambient to 55[^°]C. The chromatographic resolving power was also improved at high temperatures, especially in the case of biological samples. This was especially true for high molecular-weight branched glycans derived from human blood serum samples. The peak width decreased significantly at higher temperatures with no loss in peak area. For example, the triantennary monosialylated glycan has isomeric peak overlapping at ambient temperature under optimized gradient elution conditions. However, all isomers were resolved at temperatures higher than 45[^°]C. The resolution of isomeric peaks at ambient temperature was only 0.58, which was much lower than the 1.81 achieved at 55[^°]C. Improved resolution reduces peak overlapping, which diminishes competitive ionization in an ESI source and generates more reliable quantitative data. This high temperature LC-MS of permethylated glycans permitted the isomeric separation of glycans derived from different breast cancer cells, thus helping in better understanding breast cancer brain metastasis.

**Keywords:** Bioanalytical, Biological Samples, Carbohydrates, Liquid Chromatography/Mass Spectroscopy

**Application Code:** Bioanalytical

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
For many years now numerous studies have shown that FTIR spectroscopic analysis of cells and tissues has the potential to detect and classify disease. There have been many false dawn's, however and the reality is that this potential has not yet been realised. This is due to multiple experimental problems such as scattering artefacts and issues with reflecting substrates, which have taken the best part of a decade to identify and overcome. The major limitation now is simply one of time. If FTIR spectroscopy is to have a place in a clinical environment it must be able to deliver accurate results on a clinical timeframe. This means providing clinically significant results in minutes rather than hours or days. Typically cancer biopsy samples are formalin fixed paraffin embedded and a thin (~4 micron) thick sample is mounted on a glass slide for examination by an experience pathologist. This is a time consuming process and accuracy of results is variable depending upon the experience and expertise of the pathologist. We have previously shown that FTIR can aid diagnosis but the timescales involved in data collection and data analysis meant that FTIR was not really of practical help in a busy pathology laboratory. In this presentation we show that data collection times can be dramatically reduced using large FPAs and with judicious use of computing resources’ it is now possible to achieve near real dime data analysis. This work has been funded by the EPSRC UK.
Here, we report various examples of our latest results concerning the application of linear and nonlinear Raman microspectroscopy for clinical diagnosis. First, the unique potential of Raman microspectroscopy for an online identification of microorganisms is highlighted. The rapid identification of pathogens based in their characteristic Raman fingerprint is of great relevance for an efficient medical diagnosis (e.g. rapid identification of pathogens in urine samples) or air- and soil monitoring (e.g. identification of anthrax endospores embedded in complex matrices). The implementation of Raman spectroscopy and optical traps in a microfluidic chip allows for Raman activated cell sorting which offers large potential for an automated classification of cells like e.g. circulating tumour cells. Besides single cells, the investigation of whole tissue sections like biopsy specimens by means of Raman-microspectroscopy aiming for an early disease diagnosis will be shown. Furthermore, first steps towards in-vivo Raman spectroscopy utilizing novel Raman fiber probes for an intravascular monitoring of the artheriosclerotic plaque in living rabbits will be presented. The rather long acquisition times of Raman imaging can be reduced by utilizing non-linear Raman approaches like CARS (coherent anti-Stokes Raman scattering). In order to improve the diagnostic result CARS microscopy can be easily combined with second harmonic generation (SHG) and two-photon fluorescence (TPF) microscopy. The diagnostics potential of a compact CARS/SHG/TPF multimodal microscope as compared to conventional histopathological images will be shown for the examples of atherosclerosis and cancer.

Acknowledgements
Financial support of the EU, the “Thüringer Kultusministerium”, the “Thüringer Aufbaubank”, the Federal Ministry of Education and Research, Germany (BMBF), the German Science Foundation, the Fonds der Chemischen Industrie and the Carl-Zeiss Foundation are greatly acknowledged.

Keywords: Biomedical, Biospectroscopy, Infrared and Raman, Vibrational Spectroscopy
Application Code: Biomedical
Methodology Code: Molecular Spectroscopy
Chemical imaging, in which molecular content is obtained using spectroscopy and images are formed using microscopy, is an emerging area to characterize heterogeneous materials. We present a chemical imaging approach based on mid-infrared spectroscopic imaging that combines the spatial specificity of optical microscopy with the molecular selectivity of vibrational absorption spectroscopy. IR spectroscopic imaging is particularly attractive for the analysis of cells and tissue in that it permits a rapid and simultaneous fingerprinting of inherent biologic content, extraneous materials and metabolic state without the use of labeled probes. Recorded data are related to the structural and functional state of the biological material using computation. We describe the computational strategy and statistical considerations underlying decision-making for this modality. A combination of theory, novel instrumentation and signal processing forms an integrated approach to biochemical analyses. First, we describe attempts to automate histopathology without dyes or human input. Results indicate that a rapid assessment of tissue is possible. Applied to engineered 3D tissue models, 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 integrate imaging observations with those from conventional biological experiments to provide a complete view of cancer progression.

Keywords: Infrared and Raman, Microspectroscopy, Raman, Vibrational Spectroscopy
Application Code: Biomedical
Methodology Code: Vibrational Spectroscopy
What Lies Beneath: Probing Disease in Sub-surface Tissues Using Novel Raman Techniques

Many disease conditions are located within solid organs and are difficult to access using standard spectroscopic approaches. Advanced in novel probing of Raman scattering signals have led to the development of the field of deep Raman spectroscopy which includes both spatially offset and transmission configurations. The exploration of these techniques for probing of native disease signals from depths of many mms to cms will be outlined here. Furthermore the use of SESORS (surface enhanced spatially offset Raman spectroscopy), a novel combination of surface enhanced Raman spectroscopy and deep Raman techniques are explored here for non-invasively detecting small, deeply buried lesions using encapsulated-SERS active gold nanoparticles. This method opens prospects for in vivo, non-invasive, specific detection of molecular changes associated with disease up to depths of several cm representing significant improvement over traditionally detected Raman signals by two orders of magnitude. The disease specific signals can be achieved using uniquely tagged nanoparticles conjugated to target molecules, e.g. antibodies for production of the SERS signal. This provides the molecular specific signal which is many orders of magnitude greater than normal biological Raman signals and can clearly be easily multiplexed.

Keywords: Biomedical, Biospectroscopy, Raman, Vibrational Spectroscopy

Application Code: Biomedical

Methodology Code: Vibrational Spectroscopy
During the past years, the research group at Northeastern University has collected in excess of a quarter million infrared spectra of individual exfoliated cells from the cervix, oral cavity and the esophagus, and about 3 times as many tissue spectra from various organs. Analysis of these spectra, using supervised and unsupervised multivariate methods, has revealed accuracy in distinguishing tissue types and disease states that is better than anticipated. In particular, it appears that infrared spectral cytopathology (SCP) - the classification of exfoliated cells - can detect the presence of cancerous lesions even in cells that exhibit perfectly normal morphology, thereby offering a screening tool with higher sensitivity than classical methods. Similarly, spectral histopathology (SHP), the classification of tissue regions, can detect and classify a variety of normal tissue types, and as well as cancer types and subtypes. Similar to the SCP results where cancer field effects are detected, normal (i.e., non-cancerous) tissues in direct proximity to tumorous regions exhibit different spectral features than truly normal tissue. These results indicate enhanced sensitivity of SHP that can be used advantageously to construct expert system for the classification of tissues.

Keywords: Chemometrics, FTIR
Application Code: Biomedical
Methodology Code: Biospectroscopy
Although mechanical qualification has always been a component of the current USP Dissolution test and the USP Performance Verification Test (PVT) since 1978, the FDA has issued official guidance in January 2010 endorsing Enhanced Mechanical Qualification (MQ) as an alternative to the PVT. The implementation of the Enhanced Mechanical Qualification (MQ) for dissolution apparatus however is not as simple as performing more measurements with tighter tolerances. The MQ standards available from FDA and ASTM require additional measuring tools, component certification, laboratory controls, analyst training and documentation to implement the process successfully in the current GMP environment.

This session will cover the background of dissolution apparatus qualification, the current USP PVT, the five vital components of the enhanced MQ standards, along with proper measurements, techniques and documentation requirements for the FDA and ASTM standards.

Keywords: Dissolution, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Other (Specify)
Current Trends in Pharmaceutical Dissolution Testing

Fully Automated Dissolution Systems

For three decades, large pharma has been implementing fully-automated dissolution testing systems and a substantial amount of pharmaceutical product is tested and released using these systems. Herein we present three independent yet related topics: 1) common features of existing fully-automated dissolution systems, in an effort to better define the terminology, 2) regulatory considerations, with an eye on the USP’s plans to revise <1092>, and 3) operational considerations for running IVIVC studies, proponents of QbD, and PAT.

Keywords: Automation, Dissolution, Laboratory Automation, Pharmaceutical

Application Code: Pharmaceutical

Methodology Code: Other (Specify)
An Automated Double Dissolution System (ADDS) was developed with a Major Pharmaceutical company to address the growing need for increased dissolution testing by HPLC. The system controls the sampling and HPLC analysis of active drug components in dissolution media.

With this system, the dissolution media is circulated in a closed loop system by means of a peristaltic pump and samples are taken at the specified time intervals through the use of an innovative flow-through HPLC vial.

The ADDS software controls the whole process from taking samples from the baths to injecting the samples onto ANY HPLC or UHPLC system. Samples can also be transferred into sealed HPLC vials for storage or processing at a later date.

There are 5 different pre-program methods. Sample lists can be imported from other software data packages or created by the user. Standards and samples can be run together in bracketed sample lists. Dedicated system suitability and cleaning protocols are available.

The system is designed on a tried and tested liquid handling platform and comes complete, with a liquid handler, software, racks and sample pumps.

**Keywords:** Dissolution, Pharmaceutical, Sample Handling/Automation, Sampling

**Application Code:** Pharmaceutical

**Methodology Code:** Sampling and Sample Preparation
Presented studies describe recent developments in UV-Vis fiber optics (FO) based dissolution systems. They demonstrate benefits of [i]in situ[/i] concentration monitoring for multiple applications essential for the early drug formulation as well as late development and quality control phases. The case studies include dissolution of immediate release forms, using complex biorelevant media, the investigation of supersaturation behavior for amorphous formulations, a method for [i]in situ[/i] measurements of free API being released from nanoparticles, dissolution monitoring of dual-API formulations and a combined dissolution-permeability assay.

[i]In situ[/i] FO platform was used to obtain dissolution profiles in USP Apparatus 1 and 2 as well as to dynamically monitor concentration of APIs in 8-position mini-bath (1-20 mL). A dispensing module was used to automatically change the media during the run, e.g., changing SGF into FaSSIF or FeSSIF. Concentration of API in the presence of nanoparticles was measured using novel zero intercept method (ZIM). The Dissolution-Permeability ([micro]FLUX[circumflex O]) apparatus was used to study effect of formulations not only on dissolution, but also on the flux through artificial membranes.

The real time concentration monitoring approach eliminates filtration step with underlying liquid handling maintenance issues and uncertainties associated with non-specific filter binding. The FO based systems can also be implemented in early formulation development helping with understanding of [i]in vivo[/i] relevant processes and providing rational basis to formulation design and development. Novel ZIM method overcomes challenges associated with solid separation of nanoparticles from solution allowing [i]in situ[/i] dissolution studies of nano-formulations. [micro]FLUX apparatus assists in the investigation of a peculiar interplay between solubility, dissolution and permeability improving IVIVC models.

Keywords: Dissolution, Drugs, Fiber Optics, UV-VIS Absorbance/Luminescence
Application Code: Pharmaceutical
Methodology Code: UV/VIS
The dissolution test is a vital tool for formulation optimization, method development, formulation changes, manufacturing changes, process modifications to comply with regulatory requirements and compliance, in-vitro in-vivo correlation (IVIVC) and Scale Up and Post Approval Changes (SUPAC). One commonly overlooked parameter that can provide a wealth of information about the dosage unit under study is a visual observation of the façade of dosage forms during the dissolution process. We have used a dissolution tester (EDT-08Lx, ELECTROLAB) coupled with iDisso-06u (ELECTROLAB) for the visual evaluation of osmotically controlled bilayered extended release tablets. In addition to uninterrupted video recording of the dissolution test, the preprogrammed snapshot facility allowed comparison of the impact of process and manufacturing changes on the dissolution profile. Visual observation was complimentary to chromatographic data in evaluation of out of specification results. Hence, it is imperative to visually observe the behavior of the dosage form throughout the dissolution test run which is not a common practice with researchers using conventional dissolution apparatus. Thus, visual imaging techniques have potential applications in assessment of dissolution behavior of novel drug delivery systems. Moreover, R&D, QC and QA departments could periodically record the dosage forms undergoing dissolution for record keeping purposes as a supplement to the analytical results.

Keywords: Dissolution, Drug Discovery, Drugs, Instrumentation
Application Code: Pharmaceutical
Methodology Code: Other (Specify)
The Vertical Diffusion Cell (VDC), aka “Franz Cell,” is widely used for drug release-rate testing of semi-solids and other topical formulations. This presentation will discuss the background and development of the VDC test, current requirements and applications, the new USP General Chapters <3> and <1724> covering the testing of topical and semisolid drug products, and automated methods for VDC testing.
Photosystem I (PSI) is an integral protein that participates in the process of photosynthesis. Due to the extraordinary internal quantum efficiency and global abundance of PSI, researchers are isolating and incorporating this protein into solar energy conversion devices. Our research group has demonstrated a number of methods for depositing films of PSI onto a wide variety of electrodes. Recently, we demonstrated how significant photocurrents could be produced by depositing thick films of PSI onto a p-doped silicon electrode. Here, we will describe the benefits of integrating PSI with carbon-based materials. In particular, the design and analysis of a nano-scale photoactive electrode based on PSI and graphene will be described. Furthermore, we will discuss the benefits of implementing graphene oxide into the protein film and using reduced graphene oxide as an electrode material. Finally, the electrochemical techniques that we utilize to study this complex interface will be described.

Keywords: Electrochemistry, Electrodes, Energy, Protein

Application Code: Fuels, Energy and Petrochemical

Methodology Code: Electrochemistry
Advances in Renewable Energy Research: Devices and Analyses

Electrochemical and Spectroscopic Characterization of Sn as an Alternative Anode in Lithium-Ion Batteries

Lithium ion batteries are rechargeable batteries commonly found in notebook computers, and other portable electronic devices. Energy density requirements for larger-scale applications would require higher storage capacity from both anodes and cathodes. Current carbon-based anodes are hindered by their limited theoretical specific capacity of 375 mAh/g, as well as safety issues arising from lithium deposition. Among other group IV elements, Sn, with its relatively high theoretical specific capacity of 959 mAh/g, may prove to be a promising alternative anode material. During charge/discharge, Sn experiences volumetric changes of ~300%. This huge volumetric change causes mechanical strain leading to particle pulverization of the active material and capacity fade.

Lithium-tin alloys were electrochemically formed and investigated using cyclic voltammetry (CV) and chronoamperometry (CA). The lithiation / de-lithiation couples where identified; capacitive and Faradaic current contributions were also deconvoluted by analyzing initial (t=0 sec) and final (t=900sec) current response of individual CA measurements suggesting mass transfer processes limiting the lithiation and delithiation of Sn during charge and discharge. In-situ X-Ray Diffractometry (XRD) in a battery coin cell measured at a synchrotron source suggests the formation of various lithiated tin intermetallics. Insights into the solid electrolyte interface (SEI) and Li diffusion during the lithiation/delithiation processes were also obtained from x-ray photoelectron spectroscopy (XPS) depth profiling.

Keywords: Fuels\Energy\Petrochemical, Spectroscopy, Surface Analysis, Voltammetry
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Electrochemistry
This study reports a unique approach in which polyoxometalate-based ionic liquids (POM-IL) were synthesized and employed for the one pot dissolution and conversion of biomass. A library of four functional POM-IL compounds was synthesized using two cationic organic groups (1-butyl-3-methylimidazolium and 1-ethyl-3-methylimidazolium) and two anionic POMs (phosphotungstate and tungstosilicate). The POM-IL compound serves a dual-purpose. First, it dissolves approximately 30 wt% of cellulosic biomass in two hours at 200 °C. Second, analysis using HPLC confirmed that the POM-ILs catalyze conversion of biomass into commodity monosaccharides such as glucose and xylose. All of the prepared POM-IL compounds demonstrated dynamic thermal stabilities exceeding 300 °C and were characterized using IR and thermogravimetric analysis.
The development of renewable energy is a major topic all over the world, in response to various environmental, geopolitical and economic issues. Biomass gasification is a promising way to make renewable energy. Particularly, the 2G biomethane pathway produces syngas that may be converted into a green substitute to natural gas.

Some developments need to be achieved before reaching industrial scale. In particular, Syngas cleaning is considered as one of the most important locks to remove. Present all along the biomass gasification process, gas sampling and analysis constitute key steps in order to adjust the different process parameters, control the gas quality before the methanation step and reach finally the biomethane specifications.

Different strategies have to be used to measure tars, sulphurs and inorganic compounds. On-line measurement using e.g. micro-chromatography or laser spectroscopy is one solution. However, off-line methodology is needed when on-line measurement is not possible or when on-line quantification limits are too high. A pre-concentration step (Tar Protocol or solid phase adsorption) is necessary before quantifying the sample by gas or liquid chromatography.

Thus, the Gas Quality Section of CRIGEN - GDF SUEZ has investigated and compared many technologies for tars, sulphurs and ammonia quantification on real syngas samples. In particular, on-line measurements were performed by using the new OFCEAS technology to quantify hydrogen sulphide and ammonia. The potential of two-dimensional gas chromatography was also tested on complex syngas samples.

All these works permit to provide the most performant methodology to characterize syngas in a gasification and methanation process.

Keywords: Analysis, Energy, Sampling
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Process Analytical Techniques
Hydrothermal liquefaction (HTL) is an emerging technology that creates a bio-oil product from biomass feedstocks. This process is advantageous since wet samples can be readily processed and the potential exists to couple this process to waste water treatment. The waste water generated from the HTL conversion contains a great deal of nitrogen and phosphorus that can be potentially recycled into the algal waste water treatment process; however, it is known to be inhibitory to algal growth.

HTL conversion of Spirulina was performed. The HTL wastewater (HTL-WW) produced was processed by ultra-filtration and fractionated to facilitate toxicity assays and chemical characterization. Carbon, nitrogen, phosphorus, and sulfur mass balances were created for the HTL-WW fractions. Chronic and acute toxicity assays of the fractions were performed with Chinese hamster ovary (CHO) cells and by bacterial bioluminescence. In addition, high resolution GCMS analyses of the hydrophobic fractions were performed.

Elemental mass balances indicate that carbon, nitrogen, phosphorus, and sulfur partition to the hydrophilic fraction. Both toxicity assays indicate that the hydrophobic base fraction of the HTL-WW is the most toxic fraction. GCMS data obtained indicates that this HTL wastewater material is extremely complex with an abundance of nitrogen containing heterocyclic compounds such as pyrazines, imidazoles, pyridines, pyridinamines and additional amines. Information produced from these experiments will determine the utility of this material for bio-oil production, and will provide a baseline for water treatment options. Furthermore, these methods can be applied to other aqueous samples where water-energy interactions occur.
The chemical composition of biodiesel fuels has traditionally been determined using gas chromatography (GC) with a polar stationary phase, such as polyethylene glycol or cyanopropyl. These polar columns allow optimal separation of the fatty acid methyl esters (FAMEs) present in the biodiesels. Yet each lab uses a different column chemistry with a different set of parameters. In this work, unsupervised chemometric methods are used to associate or discriminate biodiesels from various feedstocks under various experimental conditions, such as temperature program and column polarity. Peak areas were obtained for each of the major components and evaluated using Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA).

Biodiesels in this study clustered based on feedstock type (soybean, canola, animal, etc) regardless of temperature program or column type, as long as FAME isomers were separated from one another. As such, the number and type of FAME components required to observe this clustering was investigated further. In general, the minor components in the sample did not provide improved clustering and thus did not need to be included. In addition, data from various temperature programs or column types were combined to yield similar clustering, showing potential versatility in analyzing similar samples across laboratories using different columns and column properties.

Funding for this work was generously provided by O’Brien Family Summer Research Fellowships and the Robert L. Ardizzone Fund for Junior Faculty excellence, both through the College of the Holy Cross.

Keywords: Biofuels, Chemometrics, Fuels\Energy\Petrochemical, Gas Chromatography/Mass Spectrometry
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Chemometrics
Advances in Renewable Energy Research: Devices and Analyses

Near Real-Time Determination of Inhibitors in the Production of Renewable Cellulosic Biofuels

Current approaches require 20-40 minutes to determine the levels of inhibitors produced in the conversion of cellulose to biofuels. In this paper, we demonstrate analysis times of less than a minute with conventional HPLC equipment at-line in our pilot plant. The faster analysis times are required to keep up with improved cellulosic conversion technology.

Cellulose is a renewable energy source more abundant than crude oil, environmentally friendly, and not a food source for humans. It is seemingly the “trifecta” of energy sources, but it must be converted to be useful as a transportation fuel. Cellulosic biofuel production requires four steps: pretreatment, hydrolysis, fermentation, and distillation. The last three steps are nearly off the shelf, but pretreatment remains a challenge because the same conditions used to hydrolyze the cellulose into glucose, degrade the glucose into inhibitors (furfurals). The challenge has been to find a balance between the level of pretreatment and the level of inhibitors.

Conventional pretreatment technologies require hours to produce moderately pretreated material with moderate levels of inhibitors. The National Renewable Energy Laboratory (NREL) recently announced new technology that should be able to accomplish this in 15-30 minutes at the pilot scale.

We have been producing highly pretreated material at our pilot plant with extremely low inhibitor levels in less than 10 seconds! While this is truly impressive as a pretreatment technology, it has strained the analytical lab to provide meaningful data in a timely fashion. This disruptive pretreatment technology requires a quantum leap in analytical speed. To that end, we have developed a series of robust HPLC methods to quantify the major inhibitors (furfural and hydroxy methyl furfural) down to sub ppm levels in under a minute.

This work was funded by Axion Analytical Labs, Inc., and Helios Scientific, LLC.

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Session Title: Advances in Renewable Energy Research: Devices and Analyses

Abstract Title: Near Real-Time Determination of Inhibitors in the Production of Renewable Cellulosic Biofuels

Primary Author: Lee N. Polite
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Co-Author(s): Harold M. McNair

Date: Wednesday, March 05, 2014 - After Time: 03:45 PM Room: S501a

Session # 1850 Abstract # 1850-7 Oral Sessions

Session Title: Advances in Renewable Energy Research: Devices and Analyses

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Primary Author: Lee N. Polite
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Abstract Text

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Keywords: Biofuels, Energy, High Throughput Chemical Analysis, HPLC
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Liquid Chromatography
Photosynthesis has long served as a model of efficient solar energy conversion. Billions of years of evolutionary processes in green plants and other autotrophs has created a complex pathway by which sunlight is converted into chemical energy driven principally by two of nature’s most efficient biomolecular photodiodes, Photosystem I (PSI) and Photosystem II (PSII). PSI has been widely studied for integration into bio-derived solar energy conversion devices based on its bioavailability, charge separation efficiency and robustness. Conductive polymers offer advantages as immobilization substrates by effectively “wiring” the system from enzyme to electrode, thus providing a directed path for electron transport. In preliminary work, PSI was incorporated in the conductive polymer, polyaniline (PAni), via a coelectropolymerization mechanism from a single solution of aniline and solubilized PSI. On gold electrodes, PAni-PSI composites were studied spectroscopically and electrochemically to verify both the entrapment of the protein and retention of PSI photoactivity following electrochemical deposition. In subsequent studies, PAni-PSI composites were assembled on a variety of semiconductor substrates for use in photovoltaic devices. N-doped silicon was identified as the ideal substrate for PAni-PSI deposition based on its physical and electronic properties, specifically the band alignment with PAni. Electrochemical analyses have revealed that PSI containing PAni films on n-Si yield higher photocurrents than unmodified PAni on n-Si, in the presence of an aqueous electron mediator solution. On going studies seek to transition entirely into the solid-state by constructing stand-alone photovoltaic devices through the addition of a transparent anodes, such as doped-tin oxides or graphene. This research opens future avenues for the development of bio-hybrid photovoltaics that utilize the low-cost and widely available protein, PSI, to greatly enhance solar cell efficiency.
Nanomaterials possess distinctive surface-dependent physicochemical properties and promise a wide range of applications, from consumer products to medicines. It is essential to understand their surface-dependent toxicity in order to rationally design biocompatible nanomaterials for a wide variety of applications. To this end, we have functionalized the surfaces of silver nanoparticles (Ag NPs) with three biocompatible peptides to prepare positively and negatively charged NPs. We have studied their charge-dependent transport into zebrafish embryos and their effects on embryonic development. We found that the Ag-peptide NPs passively diffused into the embryos and create charge-dependent toxic effects on embryonic development, showing that the positively charged NPs are the most biocompatible while the more negatively charged NPs are the most toxic. By comparing with our studies of the same sized citrated Ag and Au NPs, the Ag-peptide NPs are much more biocompatible than the citrated Ag NPs, and nearly as biocompatible as the Au NPs, showing the possibility of making Ag NPs biocompatible.

The work is supported in part by NSF (CBET 0507036) and NIH (R01 GM0764401).
Cardiac troponins are highly specific biomarkers for the diagnosis of acute myocardial infarction (AMI) and other chronic cardiac diseases. Current high-sensitivity troponin assays performed in a centralized laboratory have long turnaround times, while point-of-care assays have shorter analysis times but are less sensitive, thus limiting their impact in the acute care setting. Moreover, these assays are not sensitive to specific troponin degradation products that correlate to disease processes. An additional complication is the presence of anti-cardiac troponin autoantibodies found in certain healthy and diseased subpopulations that interfere with troponin assays by causing false-negative test results. These autoantibodies are also speculated to play a pathological role in cardiovascular diseases.

Silicon photonic microring resonators are highly sensitive to refractive index changes accompanying biomolecular binding to capture probes on the sensor surface. Sensitive troponin assays have been developed by using microrings functionalized with anti-cardiac troponin antibodies and secondary detection probes that incorporate signal enhancement strategies. We also have optimized assays to characterize molecularly-specific post-release troponin fragments and have also created peptide-presenting microring arrays for autoantibody-based diagnostics. Furthermore, these assays have been developed to be relatively rapid, thereby increasing their applicability to emergency and acute care settings. The resulting rapid, highly multiplexable, and fully automated microring assay formats make this approach promising for point-of-care troponin testing within a clinical environment, thus providing a diagnostic tool to dramatically improve outcomes for patients suffering from acute and chronic cardiac diseases.
Two proximity ligation assays (PLA) have been developed for leptin and insulin detection. PLAs allow for highly specific, low-volume assays that can be completed in a few hours. Specificity arises from the sandwich antibody-oligonucleotide capture method, with readout using real-time qPCR. Eliminating washing steps reduces complexity, reagents used, and assay time; and protein sample volumes can be as low as 1 [micro]L. Insulin and leptin were chosen as target proteins because they are both strongly associated with metabolic state and are altered significantly in diabetes, obesity, and metabolic syndrome. As these diseases and disorders increase worldwide, it is imperative to develop methods that help us fully understand these conditions. Extracting and testing of primary, metabolic cells from the pancreas and adipose tissue gives excellent insight into the function of these organs. Our initial testing was done with protein standards, giving LODs of 20 pM and 100 pM for leptin and insulin, respectively (60 amol and 300 amol). Adipocytes and islets of Langerhans were extracted and isolated from male C57BL/6J mice. PLA readouts from insulin secretion and leptin secretion studies are ongoing. Since PLA can also be used to assess blood plasma samples, we have acquired samples from methionine and choline deficient mice, db/db knockout mice, and wild type mice. PLA is an ideal candidate for protein quantitation in precious samples with complex matrices such as cell secretions and mouse plasma samples. The leptin and insulin PLAs developed in this work should be useful for future metabolic studies on small animal models such as mice.

Keywords: Bioanalytical, Biosensors, Protein, Small Samples
Application Code: Bioanalytical
Methodology Code: Sensors
DNA methylation is one of the most important and frequently occurring epigenetic modifications in mammalian cells. In humans, cytosine methylation is a prominent epigenetic marker, involved in gene expression, development, and disease. Mapping the sites of cytosine modification in genomes and comparing methylation patterns among individuals is crucial for understanding the underlying mechanisms by which gene expression is controlled. Here, we report a rapid and sensitive method to detect epigenetic modifications within double-stranded DNA (dsDNA) by nanopore analysis. When dsDNA strands are fed into an engineered biological protein nanopore at a fixed applied potential bias, the interactions between the dsDNA strands and the nanopore will produce characteristic current modulations, which allows the unmethylated dsDNA and methylated dsDNA to be conveniently discriminated.

**Keywords:** Bioanalytical, Biosensors, Nanotechnology, Nucleic Acids

**Application Code:** Bioanalytical

**Methodology Code:** Sensors
In Vivo Toxicology Study of Ions on Embryonic Development

Heavy metal ion exposure has been known to show adverse and lethal effects in developing embryos. However, few studies have shown the effects of silver cation and their associated anions on early embryonic development. In this study, we investigated the effects of silver cations by utilizing early developing zebrafish embryos, as an in vivo assay and model organism. Embryos were exposed to given ions throughout their entire development (120 hours-post-fertilization, hpf). The treated embryos were characterized using a wide range of assays, including observance of the biochemical responses in the embryos and chemical analysis of the silver cation solution. We found dose-dependent toxicity along with higher mortality rates and formation of deformities amongst treated embryos compared to controls. The updated results and applications will be discussed further in this presentation.

Keywords: Bioanalytical, Imaging, Toxicology, Ultratrace Analysis
Application Code: Bioanalytical
Methodology Code: Other (Specify)
Infectious diseases cause 16.2% of global deaths each year and are the second leading cause of death worldwide only after cardiovascular disease. Here, we report a real-time label-free stochastic sensing method to detect anthrax at single molecule level by using single channel recording with lipid bilayer technique. In our method, a complementary single-stranded DNA (cDNA) to a target sequence segment of anthrax is used as a sensor probe. In the absence of the target DNA, the translocation of the cDNA probe through the nanopore produces only one major type of short-lived events. In contrast, in the presence of the target DNA, these two complementary single-stranded DNA monomers would be hybridized in the solution to form a double-stranded DNA, which results in a new type of events with much longer residence times in the nanopore. The method is rapid and sensitive: picomolar concentrations of anthrax could be detected in about 1 min. More importantly, our method could differentiate the target anthrax DNA from other single-stranded DNA molecules, including those differing only by one single base. This sequence-specific detection approach should find useful application in the development of nanopore sensors for other infectious diseases.
Gold nanorods (GNRs) possess tunable optical properties that render them suitable for biomedical applications. Effective immobilization of GNRs on substrates is a crucial step towards biochip fabrication. Several disadvantages such as low stability are associated with conventional immobilization methods by solvent evaporation or electrostatic attraction. In this work, we demonstrated that a label-free optical transduction based on surface plasmon resonance shift of gold nanorod can be utilized in a chip-based format by chemisorption assembly on glass substrates. GNR self assembly monolayer (SAM) on glass surface was readily achieved by immersion of silanized substrates in nanoparticle solution. (3-Mercaptopropyl)trimethoxysilane modified substrate offers GNR deposition with maximal sensitivity to local refractive index changes, which subsequently results in better optical recognition of receptor-analyte binding. Kinetics governing the mass transport and chemisorption of nanorods from bulk to solid surface can be dynamically controlled in a predictable fashion by varying ionic strength and stabilizing surfactant concentrations. Slight aggregation induced by a low ionic strength (5 mM NaCl) can facilitate the rod chemisorptions to result in a dense, well-distributed surface monolayer. A practical application of the fabricated biochip in label-free biological detection was demonstrated for human IgG as a model with a high sensitivity at pM level. This simple and cost-effective nanosensor eliminates detection labels such as fluorescent, enzymatic, and radioactive tags.
Despite several metals necessary for human development and health, there are some metals with no known beneficial effects leaving the possibility of toxic effects due to metal ion exposure. The potential toxicity of silver cations is addressed by the chronic treatment of zebrafish ([i]Danio rerio[/i]) embryos to various concentrations of silver perchlorate (AgClO₄) solution over 120 hpf (hours-post-fertilization). Zebrafish embryos serve as an [i]in vivo[/i] assay and a model organism for the study because of their genomic homology to humans and the transparency of embryos, allowing us to directly observe phenotypic changes that may occur during the development. The lethal concentration was observed and determined based on chronic exposure and observations throughout the entire development. Embryos exhibited concentration dependent mortality. Therefore, it is proposed that exposure to a low concentration of certain metal ions, in this case silver cations, is fatal. This study will ultimately lead to further uses of the zebrafish embryos to determine specific effects of concentrations below the LC₅₀, and as [i]in vivo[/i] assays to screen biocompatibility and toxicity of other ions.

**Keywords:** Bioanalytical, Imaging, Toxicology, Ultratrace Analysis

**Application Code:** Bioanalytical

**Methodology Code:** Other (Specify)
To assess the toxicological health risk of extractable petroleum hydrocarbons (EPH) found in contaminated water and soil from leaking underground storage tanks or surface spills it is important to know how much of the petroleum compounds are aliphatic and aromatic. The aliphatic compounds (C9-C36) pose less of a health risk as compared to the polyaromatic hydrocarbons (PAHs) in the C11-C22 range. The 2004 method developed by the Massachusetts Dept. of Environmental Protection (MADEP) fractionates the extracts into the two hydrocarbon classes using manual solid phase extraction (SPE) with 5 g of silica gel packed into a 20 mL cartridge. Aliphatic compounds are eluted in the first fraction with hexane while the aromatics are collected in the second fraction with methylene chloride. The fractions are concentrated down to 1 mL and analyzed separately by GC-FID. The manual fractionation process is prone to poor recovery and reproducibility if proper technique and care of the cartridge is not followed. Automation of the fractionation step reduces operator error and variation while reducing time and consumption of solvent waste. A 6 mL cartridge filled with 2.5 g of silica gel was developed to interface with the automated solid phase extraction instrument. To eliminate hydrocarbon extractables from the polypropylene cartridge, a Teflon coated variety was selected. In this presentation the optimization of the instrument configuration and method on this silica-gel cartridge will be reviewed. Performance data from the automated method as applied to artificial and real samples for % recovery and the quality of the fractionation will be shown.
Hydraulic fracturing, the fracturing of a rock by a pressurized liquid, is a well stimulation technique in which typically water is mixed with sand and chemicals, and the mixture is injected at high pressure into a wellbore to create small (<1mm) fractures in order to maximize fluid removal and well productivity. While this once-very diffused technique makes accessible big amounts of formerly non-accessible hydrocarbons, the dissolved gases have become a controversial environmental and health matter with some countries completely banning the practice. Public outcry over preservation of water quality has led the U.S. EPA and other state agencies to investigate the impact of hydraulic fracturing on the quality of environmental waters. Some procedures for testing waters for dissolved gases through static headspace sampling exist already, like RSK 175 standard operating procedure, but since the target compounds are light hydrocarbons from methane through propane, are extremely volatile, a closed sampling system is required. A high throughput test method using robust, automated and relatively inexpensive instrumentation like static headspace and gas chromatography with flame ionization detection is used in this paper, and data will be shown for the quantitative determination of dissolved gases in ground, waste and drinking waters.
Oil and Grease is a relatively simple measurement with a number of environmental applications. The parameter can be measured in different ways and is used around the world in a variety of regulatory applications and consensus standards. For example, as a method defined parameter, it is used quantitatively to assess wastewater treatment plant performance and permit compliance. In evaluating potential site contamination it may be used as an inexpensive tool to screen soil for oily waste contamination in order to eliminate the need to subject “clean” samples to more expensive and more time consuming detailed chemical analyses.

This paper will trace the development of Oil and Grease determination methodology and look at how it is used. The oil and grease measurement methods used in the US, Europe and Asia will be compared. Recently developed techniques for measuring this parameter that are less costly and environmentally greener will be discussed.

Keywords: Characterization, Environmental/Water, Extraction
Application Code: Environmental
Methodology Code: Other (Specify)
This study focuses on air quality analysis by detecting trace levels of Formaldehyde and BTX, which is a common acronym in petrochemical industry that stands for benzene, toluene and the three xylene isomers. These gases occur in urban and indoor air. It can outgas from commodities, cleaning agents, printings, paints and wood panels, but also tobacco smoke contributes largely to the indoor BTX concentrations. Formaldehyde and BTX can affect human health and cause discomfort, irritation of the eyes, nose, and throat, problems with nervous system and at elevated concentrations finally death. Trace level monitoring of these gases is important when analyzing the quality of ambient or indoor air.

The high sensitivity in a wide dynamic range of the proposed system is achieved with a silicon MEMS cantilever sensor coupled with an optical readout system [1] and laser source, which is operating at the fundamental vibrational absorption wavelengths of the analyzed gases. High selectivity is achieved by measuring the infrared spectra of the sample gas utilizing widely tunable laser sources such as optical parametric oscillator (OPO) and Quantum Cascade Lasers (QCL). The combination of these technologies allows the development of a handheld size device, which is required by the industry.

Compact and fast measurement system based on a novel combination of cantilever enhanced photoacoustic spectroscopy and compact optical parametric oscillator (OPO) was applied to measurement of BTX traces with detection levels below ppb [2] and combination of photoacoustic detector with mid-IR QCL was applied for selective and sensitive formaldehyde analysis with below ppb sensitivity [3].

In retro-engineering techniques aimed at re-formulating and improving an existing food product, it is important to get detailed and precise sensory information about target competitive products taken as a benchmark. In this study, it is proposed to assess the flavour, taste and visual aspect of sausages using respectively an electronic nose, an electronic tongue and a visual analyser. Six batches of the company product are compared to two competitive brands of sausages. The fast gas chromatography based electronic nose shows little differences on the odor profiles of the three brands except that the two competitive sausages contain terpenes which could indicate that higher proportions of spices and herbs were added in these formulations, with an aromatic profile similar to fennel. The three brands of sausages show very different taste profiles according to the electronic tongue analysis. A relative ranking on the intensity of sourness, sweetness, saltiness and umami highlights that the company sausages were slightly less acidic, less sweet, less salty and less umami than the competitive products.

The visual analysis of colors and shapes is achieved with a camera-imaging system. The differences of aspect between the three brands are mostly correlated with brown colors intensity. The appearance of the company product is closer to competitor B whereas competitor A is quite different. The data produced by the sensory analysis instruments give an accurate and objective comparison of the different products and provide useful information to help re-develop the sausage recipe in order to get closer to the target.

Keywords: Food Science, Gas Chromatography, Imaging, Sensors
Application Code: Food Science
Methodology Code: Chemometrics
Organic acid profiles in fruit juices are important in the beverage industry to characterize flavor, maintain product quality, and to meet labeling requirements. To analyze organic acids and anions of strong acids, such as chloride and sulfate, which are also present in beverages, ion chromatography with suppressed conductivity is the ideal analytical method. Unlike the anions of strong acids which are fully ionized, organic acids are weakly ionized and can exhibit lower conductivity responses versus concentration than the strongly ionized anions. The new Thermo Scientific Dionex QD Charge Detector promotes complete disassociation of many weakly disassociated compounds by drawing a current at a fixed potential. As a result, the charge responses of singly-charged organic acids and doubly-charged and triply-charged organic acids are proportionally higher than conductivity.

Here we demonstrate separations of organic acids on a 4-µm particle size, capillary anion-exchange column. Four µm particle columns produce high efficiency separations but also have higher system backpressure, and therefore can only be used on a high-pressure capillary IC system. Capillary IC at µL/min flow rates is always on and ready for analysis and requires only 5.2 L/yr of deionized water. The results show comparably higher QD response for organic acids compared to chloride and sulfate. Additionally, use of CD/QD ratios to assess peak purity is demonstrated, thereby improving reporting accuracy.

Keywords: Beverage, Capillary LC, Detection, Ion Chromatography
Application Code: Food Science
Methodology Code: Liquid Chromatography
Monitoring natural products is important in the food and beverage industry. Understanding the inherent variations in raw materials can assist in process optimization. One of the primary ingredients used in beer brewing, hops are a natural product of particular interest. These leafy green flowers provide the characteristic bitter taste in beer and also contribute complex fruity, tangy, and piney flavors. Bitterness in the finished product is measured by extracting acidified beer with isooctane. Iso-alpha-acids, responsible for bitterness, partition into the isooctane layer and are measured with UV-Vis spectrometric detection at 275 nm. The resulting measurement is reported as the International Bitterness Unit (IBU), as defined by the American Society of Brewing Chemists. Dry hopping, the addition of hops after the fermentation has started, often results in a measurable increase in absorbance at 275 nm without a corresponding increase in perceived bitterness. This increase in IBU between the pre- and post-hop addition is referred to as the “dry hop index.” Here, we develop a gas chromatography with time-of-flight mass spectrometry (GC-TOFMS) based method to explore the dry hop index as it relates to iso-alpha-acid concentration, matrix effects of non-bittering analytes, and hop quality.
An analytical method about the determination of natural vitamin E and the benzopyrene (BaP) in the natural vitamin E raw materials has been developed by High Performance Liquid Chromatography/Fluorescence Detector (HPLC/FLD). After the purification and enrichment by Cleanert Bap-3 SPE column series connection with Cleanert Silica SPE column, the natural vitamin E and benzopyrene can be successfully separated by isocratic elution (acetonitrile:water=80:20) on Venusil ASB C18 column. The spiked recovery of BaP in natural vitamin E samples were in the range of 81.3-87.0%, and the calibration curves has shown good linearity ($r=0.9996$). The LOQ of this method for benzopyrene was 0.5 μg/g, and the detected results of benzopyrene in the natural vitamin E was consisted with GC-MS.

**Keywords:** Amino Acids, Food Science, HPLC Detection, Liquid Chromatography

**Application Code:** Food Science

**Methodology Code:** Liquid Chromatography
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<td>Abstract Title</td>
<td>The Importance of GC-TOFMS and GC-HR-TOFMS for Flavor and Off-Flavor Analysis for Packaging Related Issues</td>
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Abstract Text
Advances in sample preparation techniques prior to GC-MS analysis and the complicated matrices of the samples tested often result in a plethora of chromatographic peaks. It is difficult to be confident that coelution of peaks is not occurring and that all peaks are accurately identified. This presentation will give examples of how often coelution problems can occur when analyzing complex samples, like extracts from packaging materials, and will show how the Pegasus HT TOF can be used to resolve “hidden” peaks, resulting in higher quality spectra and more accurate peak area determinations. Furthermore, the capability of ChromaTOF software to provide results quickly will be discussed. The usefulness of accurate mass information from high resolution TOFMS detection for determining unknowns and confirming identification in flavor/off-flavor analysis will also be illustrated. In addition to packaging, other examples illustrating the importance of peak deconvolution in flavor/off-flavor analysis for different food samples will be presented.

Keywords: Agricultural, Flavor/Essential Oil, Food Science, Gas Chromatography/Mass Spectrometry
Application Code: Food Science
Methodology Code: Gas Chromatography/Mass Spectrometry
Surface Plasmon resonance (SPR) has been received attention as ultra-highly sensitive mass transducer. First, we report here the portable SPR sensor system employs the angle-fix configuration. It is able to detect mass change at the interface in pico-gram order. The sensor surface was fabricated using self-assembly method of functionalized alkanethiol on Au. It was found that the alkanethiol domain structure highly affected on the sensitivity of immunoassay. Secondary, we propose the new kinetics equation for the indirect competitive inhibition immunoassay that is to detect a small target molecule. Based on our kinetics equation, the optimization of the sensor surface structure will be discussed. Most of immunoassays employ the bioactive material (antibody or antigen)-polymer conjugate on the sensor surface. Although a polymer conjugate shows very high SPR signal response, the sensitivity is somehow low. The reason is that the bioactive materials randomly bind to the polymer network. In order to control the orientation and the surface concentration of bioactive materials, self-assembled monolayer of functionalized alkanethiol is used for immobilization of bioactive materials onto sensor chip surface. In this study, we will compare the sensitivities of those immunosurfaces. In addition, the strategy of the multi target detection of agonists such as clenbuterol, ractopamine and salbutamol will be reported. The immunosurface structures were characterized by electrochemical methods, scanning tunneling microscopy (STM), X-ray photoelectron spectroscopy (XPS), and FT-IR RAS.

Keywords: Biosensors, Characterization, Electrochemistry, Electrode Surfaces
Application Code: Food Science
Methodology Code: Portable Instruments
Variability introduced by the sequence of unit operations in food processing directly influences the compositional and sensorial properties as well as the safety of the final food products. Conventional strategies of Quality Assurance can be effective, but are expensive and not flawless. Therefore food industry is trying to shift towards a novel holistic concept, namely “Quality by Design” (QbD) inspired by the PAT, which requires that quality of the food products should be incorporated into process development and design, not by post-production quality testing. This requires systems that enable continuous analysis and control of processes, including reliable non-destructive real-time sensing and multivariate data analysis of both raw materials and in-process materials. Most of the interesting target volatiles in food safety and process control applications have a characteristic spectrum in the Mid-IR range such as Furan, Acrolein, and Dimethyl Sulfide.

A novel sensor for on-line and real-time monitoring of volatiles in food/beverages is introduced. Recent developments in improving the performance of the photoacoustic detection scheme have led to the introduction of optical cantilever microphone and compact tunable laser sources operating at the fundamental vibrational absorption wavelengths of the analyzed gases. The main part of the optical cantilever microphone is an ultra-sensitive silicon cantilever sensor, combined with interferometric readout technology. Cells equipped with a Cantilever microphone have proven to be 100 times more sensitive than more conventional photoacoustic cells with capacitive microphones. A further benefit of the optical readout technology is the fact that it can be operated at elevated temperatures. The developed system is tested in three case studies: bread processing, potato chips frying and brewing.

The research work is partly funded by the European Commission within the FP7 project called MUSE-Tech.

Keywords: Food Science, Photoacoustic, Process Analytical Chemistry, Spectroscopy
Application Code: Food Science
Methodology Code: Vibrational Spectroscopy
The talk will discuss the supply shortage of helium and peoples decision to switch to hydrogen as an alternate source of carrier gas. The shortage has almost gone away but the costs and concerns over the cost and availability of the gases we use for chromatography still exist. We can do something about our usage. Discussed will be the newest and best approaches to handling your gases. The best and most efficient ways to get gases to the chromatograph from your source (cylinders, tanks, and other hook ups) without loss to leaks or possible contamination. The very process of how to change cylinders is possibly the single most costly and damaging step taken in the operation of a chromatograph. Other problem sources are the regulators, fittings, and tubing connections and purifier’s can be problems for contamination and loss of gas. With an optimized gas supply, your chromatographic system will show many improvements and last longer. These changes can save you significant amounts of gas and deliver it at higher purity than you currently receive. It will be shown that improvements available to you can save you twenty to thirty percent of the gases used while delivering a better product to the chromatograph. This will lead to better and more stable baselines and the higher purity gas will lead to longer column life. The fewer times you have to take a system down to change columns the more samples can be run and this leads to tremendous savings in the laboratory.

Keywords: Capillary GC, Gas, Gas Chromatography, Gas Chromatography/Mass Spectrometry
Carrier gas is an important component in generating a gas chromatogram. The analytes must be transported down the column by the carrier for them to fully interact with the stationary phase to separate the different species. The choice of carrier gas is limited by demands of the measurement. These requirements include:

- Inert, to avoid any chemical interaction with sample analytes and column degradation
- Appropriate for detector used
- Optimum flow rate to minimize peak width while shortening run time
- Readily available
- Pure
- Inexpensive

In the early days of gas chromatography, nitrogen and helium were commonly deployed as carrier gas, as they met all of the requirements when used with packed columns and worked quite well for most applications. Advent of capillary columns in the late 1970's generated a mass conversion to helium to take advantage of the improved analyte transfer between the capillary stationary phase and the carrier gas with the narrower columns. Now with supplies of helium dwindling and its price escalating, hydrogen is being considered often as a direct replacement.

This presentation provides a discussion of effects on the chromatographic process with a switch to hydrogen carrier gas, including changes in column performance, impact on calibration of pneumatic components, compatibility with detectors, and potential alterations in regulatory protocols.
Many labs are often asked to do more with less, whether this is to reach lower detection limits with current instrumentation or to do more work using fewer resources. Implementing a large volume injection (LVI) technique is one way to achieve either or both of these goals. LVI can improve detection limits by injecting more analyte on column. Alternatively, if less sample is processed, a larger injection can still result in the same amount of sample on column. LVI is typically performed using a specialized inlet though and has required an initial investment and/or instrumentation upgrades. This has made LVI out of reach to most labs. Significantly larger injections can still be performed using traditional split/splitless inlets though if using the correct parameters and hardware.

This work explores the requirements and limitations for LVI using traditional split/splitless injections. In some instances, injections up to 25 µL can be performed using existing hardware. This can significantly improve detection limits or dramatically reduce the volumes required for sample preparation. The effects of liner selection, solvent selection, column configuration, and oven temperatures are shown using commonly run EPA methods like PAHs and other Semi-volatiles.

Keywords: Environmental Analysis, Gas Chromatography/Mass Spectrometry, Sample Introduction, Trace Analysis
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<td>Abstract Title</td>
<td>How to Manage Helium Shortage? Let's Use Hydrogen to Measure THT in Natural Gas with Micro-Chromatographs</td>
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**Abstract Text**

Helium is no longer as available and cheap as it used to be. For process control of odorisation of natural gas (tetrahydrothiophene), GDF SUEZ branches uses helium as carrier gas for micro-chromatography. The precision of this detector depends on the conductivity of the carrier gas. For that reason we decided to test this device with hydrogen. The analytical method is now optimized: sampling time, pressure and temperature column. This method gives good repeatability and precision, the response is linear in the range of concentrations considered. We also tested Argon and Nitrogen to compare.

**Keywords:** Analysis, Chromatography, Gas Chromatography, Trace Analysis

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

**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 room air 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, push the sample holder into the open probe oven for its thermal vaporization and have the results 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 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 requires the simple and low cost mass spectrometer of GC-MS.

The operation of the Open Probe fast GC-MS will be demonstrated with the 20 s separation time and 30 s full analysis cycle time of heroin in its street drug powder. Similarly, cockroach repeller liquid residue on tomato was analyzed in 30 s and trace TNT on human hand was analyzed in 40 s with NIST library identification of TNT.
Design and Fabrication of Multiplexed Plasmonic Nanorod Biochip for High Throughput Biological Assay

Abstract Text
Surface plasmonic sensors are highly specific and sensitive that can be used to monitor refractive index changes caused by molecular interaction. The wavelength dependence of anisotropic gold nanorods (GNR) provides an excellent multiplexing capability. These properties have attracted significant interest in the development of nanorod based plasmonic biochip for biomedical application. In this work, we demonstrate a nanochip biosensing technology that employs surface plasmonic resonance shift of GNR towards multiplexed and high throughput screening of bimolecular interaction for early stage medical diagnosis. This high throughput nanorod chip was achieved by chemisorptions assembly of GNRs on 96 well plates using the linker of (3-Mercaptopropyl)trimethoxysilane, which forming a evenly distributed monolayer of GNRs on substrate. Three different aspect ratio of GNRs (aspect ratio, 2.1, 3.2 and 4.7) and three model antibody (goat anti-human IgG, goat anti-mouse IgG, goat anti rabbit IgG) were chosen in this study. By linking the Fc portion of antibody toward gold naorods surface, we demonstrated directional conjugation onto GNRs to form functional biosensor. Multiplex sensing was then developed by the distinct response of the plasmon spectra of the different aspect ratio of GNR. Simple and convenient uv-vis spectrophotometer was used to monitor the target binding events based on the sensitive refractive index change. This high throughput label free biochip can be used for quantitative detection of different biomolecule simultaneously with a high detection sensitivity, low detection limit and minimal cross-reactivity. This technique opens a new way in developing a user-friendly, point-of-care optical biosensor for high throughput and multiplexed biological detection.

Keywords: Biosensors, High Throughput Chemical Analysis, Nanotechnology, UV-VIS Absorbance/Luminescence
Application Code: High-Throughput Chemical Analysis
Methodology Code: Sensors
**Abstract Text**

The cost and complexity of current methods for arsenic monitoring in ground and surface waters provide limited sampling opportunities. Due to the prevalence of As in ground and surface waters and its deleterious effect on human health, electrochemical remote automated sensors could potentially become a valuable tool for assessing arsenic levels in such waters. The most common species of arsenic in ground and surface waters are the inorganic forms As(III) and As(V), with As(III) reportedly 25-60 times more toxic as As(V). We will describe the application of subtractive anodic stripping coulometry (SASC) for calibration-less determination of As(III) in water samples. The coulometric sensing platform consists of a micro-fabricated Au electrode on a SiO2 substrate and a stopped-flow, thin-layer µL-volume refillable flow-cell. Recent findings that coulometric analyses can be reliably performed without any need for sample pre-treatments such as acidification or oxygen removal will be presented. Using SASC, the sensor is presently capable of measuring concentrations as low ~500ppb (RSD’s <5%). These measurements are possible not only in the absence of standard solutions for calibration, but also without the use of a blank solution for subtracting background signals. Combination of this approach with low-cost field-deployable electrochemical instrumentation is promising for the development of long-term unattended field monitoring networks. The EPA and WHO recommended limit of arsenic is 10ppb. Further reduction in our sensor’s detection limit is desirable and preliminary results show that significant improvements are possible via alternative electrode designs such as micro-electrode arrays and modification of the SASC waveform and parameters. This approach will be presented in detail along with the special considerations arising from the use of a thin-layer-cell configuration. Determination of As(III) in the presence of potential interferents will also be presented.

**Keywords:** Electrochemistry, Lab-on-a-Chip/Microfluidics, Stripping Analysis, Water

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

**Methodology Code:** Electrochemistry
The global helium shortage and price increase cause more and more analytical laboratories employing gas chromatography to re-assess their consumption patterns of this non-renewable noble carrier gas. The existing solutions to this problem include changing analytical column, migrating to another carrier gas (e.g. hydrogen) or passively reducing the helium consumption by reducing its usage during analytical runs or switching the GC or GC-MS over to nitrogen during the longer idling periods. In all these cases a considerable amount of time is spent developing new methods or wasting daily the time needed for the instruments to come back to normal operations. A new, innovative approach to the conservation of helium carrier gas will be presented, comprised of separation of the flows inside the standard split/splitless injector. While preserving the analytical GC column flow with helium, the septum purge and the split flows are maintained with another inert gas like nitrogen - even during the analytical run. On average, the helium consumption is reduced such that a standard cylinder of compressed helium gas can last 3-6 years vs. 3-6 months without and changes in the analytical methods or conditions. This new patented technology for saving Helium will be presented, along with the data validating its use; the additional practical benefits introduced to the quality of the chromatographic data will be also discussed.

Keywords: Capillary GC, Gas Chromatography, Instrumentation, Laboratory
Application Code: High-Throughput Chemical Analysis
Methodology Code: Gas Chromatography
New Derivatization Reagents to Optimize Retention and Response for Quantitative Analysis by LC-ESI-MS/MS

Several novel cationic sulfonyl chloride derivatizing reagents were synthesized from commercially available zwitterionic surfactants and evaluated in LC-ESI-MS/MS experiments. Signal enhancements as large as 300% were observed by increasing the alkyl chain length of the derivatizing reagent. By preparing reagents of varying hyrophobicity, retention of derivatized analytes on reversed phase columns was adjusted, granting the ability to minimize signal suppression due to co-eluting matrices. Excellent sensitivity, linearity and reproducibility were observed in LC-MS/MS methods. A detection limit of 25 fg/mL is reported for derivatized p-cresol and S/N > 400 was observed for derivatized estradiol extracted from an aqueous matrix at a concentration of 10 ng/mL. Detection limits and retention profiles for derivatized analytes extracted from various biological matrices is presented illustrating the applicability for trace bioanalytical quantitation of small molecules that show poor inherent signal intensity in LC-MS/MS methods.

Keywords: Biological Samples, Liquid Chromatography/Mass Spectroscopy, Tandem Mass Spec, Ultratrace Analy
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Protein glycosylation is one of the most important and common protein modifications in biological systems. It is involved in a wide range of cellular events such as immune response, cell division and cell-cell communication. Aberrant protein glycosylation is directly related to several diseases, including diabetes, infectious diseases and cancers. Due to the heterogeneity of glycans and low abundance of glycoproteins, it is extraordinarily difficult to comprehensively identifying protein glycosylation sites by MS. Based on the chemical bond differences between O- and N- linkages of sugars, we have developed a novel method of integrating chemical deglycosylation and MS for comprehensively profiling N-glycosylation sites in complex biological samples. With the optimal conditions, O-linked sugars were removed and only the innermost GlcNAc was left and served as a common mass tag for large-scale site identification by MS. Compared to the enzymatic methods, the current chemical method is more cost-efficient, generic and effective. Comprehensive analysis of protein glycosylation will aid us in a better understanding of glycoprotein structures, activities, and functions.

Keywords: HPLC, Mass Spectrometry, Proteomics, Sample Preparation
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Nanosecond pulse length laser desorption combined with 10.5 eV postionization has been demonstrated for molecular imaging of intact biological samples [1]. Femtosecond pulse laser desorption can be used for precision removal of biological material from intact samples and, potentially, depth profiling, a capability not available to nanosecond pulsed desorption [2]. Femtosecond laser desorption with time-of-flight mass spectrometry is used here to measure survival yields (SY) of direct ions [3] and 10.5 eV postionized neutrals (post-ions) [4]. The thermometer molecule 4-chlorobenzylpyridinium bromide spiked into polyelectrolyte multilayers (PEM) (chosen to model a biological matrix [5]) and animal tissue served to estimate the internal energies (E\text{int}) of ablated species. Ablation was conducted in a scanning mode under pulse overlap degree corresponding to values of 0.9 and 0 commonly used in experiments (“high” and “no” overlap, respectively). SY of direct ions sampled from PEM increased from 0.2 to 0.5 as the laser fluence increased from 0.12 to 0.94 J/cm\textsuperscript{2}. Comparison with published RRKM calculations [6] indicated that the E\text{int} of direct parent ions varied from 5.5 to 5.8 eV. The ablation laser fluence threshold for the appearance of post-ions was significantly higher than for direct ionization while analysis yielded SY of 0.8 corresponding to E\text{int} of 5.2 eV under no-overlap sampling conditions. Analysis of direct ions from animal tissue was not possible while obtained SY and E\text{int} of post-ions under both overlap regimes were 0.5 and 5.5 eV respectively. The implications of these results are discussed for mass spectrometric imaging using femtosecond laser desorption.


Keywords: Imaging, Laser Desorption, Mass Spectrometry, Ultra Fast Spectroscopy
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Controlled Proteolysis in Trypsin-modified Membrane to Obtain Large Peptides for Mass Spectrometry

Examination of large (2000-15000 Da) proteolytic peptides with mass spectrometry, also known as middle-down analysis, is an important approach for protein characterization. However, the first step in such investigations, controlled generation of large peptides, is often challenging. Limited protein digestion during short residence times in trypsin-modified membranes provides a new strategy to obtain tryptic peptides for middle-down analysis. Large peptides appear when the protein residence time in the membrane is on the millisecond level, and peptide size depends on the membrane pore diameter and the local trypsin concentration. Among the peptides of apomyoglobin generated using a residence time of 5 ms in a membrane with 1.2 micrometer pores, two peptides can cover the entire protein sequence. In contrast with a residence time of 5 s, full sequence coverage requires 8 peptides. Digestion of alpha-casein in a nylon membrane with 5 micrometer pores yields several peptides with masses above 10 kDa and shows a more limited proteolysis than digestion in membranes with 1.2 micrometer and 0.45 micrometer pores. Future work will study high-throughput generation of large peptides for protein identification and characterization of post-translational modifications by mass spectrometry.

Keywords: Mass Spectrometry, Membrane, Method Development, Proteomics
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Nanoelectrospray ionization (nanoESI) is a soft ionization technique for mass spectrometry that uses an applied voltage to spray solution from emitters with orifice diameters of 50-100 micrometers. Work presented here demonstrates the ability to perform nanoESI from capillaries pulled to orifice diameters less than 100 nanometers and looks at the fundamentals of nanoESI from these small emitters. Smaller nanospray orifice dimensions can 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, the fundamentals behind our nanoESI technique are examined with a focus on flow rate, current, and analyte influence on electrospray and the resulting spectra. Our results demonstrate that use of nanopipettes as nanospray ionization emitters is a promising new approach for ultra-trace biological mass spectrometry.
Negative ion electron capture dissociation (niECD; discovered in our laboratory) involves peptide anions capturing 3.5-7.5 eV electrons, generating charge-increased radical anions that undergo dissociation analogous to conventional ECD. We proposed that gaseous zwitterionic structures are necessary for niECD with electron capture occurring at or being directed by a positively charged site. To validate this mechanism, niECD was applied to three sets of synthetic peptides. Peptides were designed with varying numbers and positions of charges (Figure 1). Because it contains multiple acidic sites for deprotonation and the N-terminus for protonation, P1 should have high probability of being zwitterionic. For P2-6, the probability of zwitterionic structures was decreased by gradually replacing acidic residues with neutral ones. Consistently, niECD efficiency decreased from P1 to P6. To manipulate the basic sites, P7-12 were synthesized with lysine replacing the C-terminal glutamine. With more available protonation sites, zwitterions should form more readily. Similar to P1-6, the niECD efficiency dropped as the number of acidic sites dropped. However, unexpectedly, niECD performance was worse for P7-12, possibly due to a salt bridge forming between the lysine side chain and the C-terminus. In P13-18, the basicity was further enhanced by converting the lysine side chains to guanidinium groups. The same decreasing niECD efficiency trend was observed. However, the efficiency was even lower than for P1-12, indicating that interaction may also occur between the guanidinium group and the C-terminus, and that a more mobile proton may be necessary. Overall, all three datasets follow the same trend and support our zwitterion mechanism.

Funding: NSF CHE-1152531

Keywords: Ion Cyclotron Resonance, Mass Spectrometry, Proteomics, Tandem Mass Spec

Application Code: Bioanalytical

Methodology Code: Mass Spectrometry
Individual cells within a population often vary significantly. MALDI-mass spectrometric analysis at small scales can be a valuable tool for determination of cellular processes. Typically, the spatial resolution of MALDI-MS is limited by laser spot size which limits the ultimate resolution that can be achieved. The use of nanopipette probes as a diagnostic tool for nanoscale sensing has been demonstrated in a number of venues, and the size scale is commensurate with cellular or subcellular measurement. Combining these two existing techniques, we have developed a sampling technique for collection of high spatial resolution with MALDI mass spectrometry. Moreover, we will discuss strategies for sample clean-up to enhance chemical information obtained.

**Keywords:** Bioanalytical, Mass Spectrometry

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
Analysis of volatile organic compounds (VOCs) in breath holds great promise as a novel non-invasive diagnostic tool. Clinical application, however, is hampered by problems arising from actual or recent uptake of exogenous substances, especially in the clinical environment, and from quickly and abruptly changing VOC profiles. Real time measurements can help to address these issues.

Therefore, a PTR-ToF-MS was adapted for clinical breath analysis. For reasons of medical safety, the PTR-ToF-MS instrument could not be positioned at the bedside. Hence, a 6m long heated silcosteel transfer line connected to a sterile mouthpiece was used for breath sampling. Breath from mechanically ventilated patients was measured continuously for one hour directly after cardiac surgery. Room air and exhaled air from 32 volunteers was analyzed in the post anesthetic care unit (PACU). In addition, ambient air in the PACU was analyzed continuously over 7 days.

Breath resolved measurements in mechanically ventilated patients and spontaneously breathing volunteers were possible using a time resolution of 200 ms. Detection limits were in the high pptV / low ppbV range (e.g. 0.4 ppbV for sevoflurane). Changes of ventilation-perfusion-ratio, cardiac output and application of intravenous drugs were mirrored by VOC concentrations (e.g. isoprene) in the breath of mechanically ventilated patients. Exhaled concentrations of sevoflurane in the PACU strongly depended on background concentrations and a good correlation with room air concentrations was observed (R²=0.94). Endogenous acetone in contrast did not show such a dependency.

PTR-ToF-MS adapted to clinical conditions is a powerful tool to address fundamental as well as practical questions in clinical breath analysis.

Keywords: Biomedical, Mass Spectrometry, Time of Flight MS, Volatile Organic Compounds

Application Code: Biomedical

Methodology Code: Mass Spectrometry
Uranium detection research is of sustained interest in nuclear technology and safeguards, thus placing a high need for improved instrumentation. A variety of techniques have been employed to examine uranium, both chemically and physically. Alpha spectrometry, gamma spectrometry and mass spectrometry (MS) are the most common techniques used for analyzing uranium. Detection of uranium in solution using MS is based on either the aqueous chemistry dominated by the uranyl dication, UO\[\text{II}\] (e.g., electrospray ionization-MS) or complete atomization of the sample (e.g., inductively coupled plasma-MS). Current existing methods do not provide adequate molecular information for positive ion detection mode, which is often affected by the solvent composition.

This laboratory has developed a liquid sampling-atmospheric pressure glow discharge (LS-APGD) as a low power and cost efficient ionization source for mass spectrometry. The glow discharge is sustained between the surface of electrolytic solution, introduced through a glass capillary (0.28-mm i.d.) housed inside a metal capillary (1-mm i.d.), and a stainless steel counter electrode mounted at a 90° angle. The source is interfaced to a mass spectrometer to analyze three uranyl compounds: uranyl nitrate, uranyl chloride, and uranyl acetate. Direct sample injection (50µL) of each compound into the flowing electrolyte steam (at 25µL/min) was introduced to the LS-APGD. The resultant spectra provide both atomic and molecular information. Results showed that the molecular structure was the main factor that influenced the ion formation in both positive and negative ionization modes. The limits of detection have also been established for all three compounds.

Keywords: Elemental Analysis, Elemental Mass Spec, Mass Spectrometry, Plasma
Application Code: General Interest
Methodology Code: Mass Spectrometry
Biogenic amines, such as dopamine, are of great interest as they serve a central role in neuronal communication and have been linked to many neurological disorders. The ability to detect, characterize, and quantify these species allows for improved understanding of neuronal signaling and disease states. However, these compounds can be difficult to detect at low concentrations in mass-limited complex biological samples. Additionally, they required time-consuming separation schemes. In this work, biological samples from [*Drosophila melanogaster*](https://en.wikipedia.org/wiki/Drosophila_melanogaster) are prepared and extracted using a benzoyl chloride labeling reaction, followed by a liquid-liquid extraction procedure. Benzoyl chloride labels phenols, and primary and secondary amines, making it a promising derivitizing agent for biogenic amines (which tend to label between one and three times). The reaction results in chemically stable compounds with increased ionization efficiencies. By employing a liquid-liquid extraction method to clean up labeled samples, we have easily coupled the labeling strategy with MALDI-MS detection. Liquid-liquid extraction using diethyl ether is efficient, low cost, and rapid. The extraction allows for purification of small volume, crude samples without a lengthy separation. When coupled to MALDI-MS, this method is high-throughput and sensitive. The labeling strategy also enables the distinction of isobaric species such as dopamine and octopamine due to differential labeling by benzoyl chloride. Beyond characterization and optimization of the method, results from multiple model systems in [*Drosophila melanogaster*](https://en.wikipedia.org/wiki/Drosophila_melanogaster) are presented.

**Keywords:** High Throughput Chemical Analysis, Laser Desorption, Mass Spectrometry, Neurochemistry

**Application Code:** Neurochemistry

**Methodology Code:** Mass Spectrometry
The three-dimensional structure (or shape) of a cell to cell signaling molecule is a critical aspect of binding to its receptor. Neuropeptides are signaling molecules in the nervous system that modulate neuronal activity. They are derived from cleavage and modifications of larger protein precursors, which are known to be ribosomally translated using only L-amino acids. However, D-amino acid-containing peptides (DAACPs) exist, forming as a result of a post-translational isomerization of a single amino acid residue. Although subtle, this modification produces pronounced effects in the peptide’s shape and bioactivity. DAACPs exist in vertebrates and as molluscan neuropeptides. Isomerization does not alter the molecular weight of a peptide and so is not straightforward to characterize via mass spectrometry (MS); in fact, most DAACP discovery has been based on bioactivity or homology. With MS leading the discovery of novel neuropeptides, it may be that many DAACPs have not been characterized. Thus, it is important to develop methods for DAACP discovery in a sensitive and non-targeted manner, to identify the extent to which peptide epimerization is present throughout the metazoa. Toward this goal, we study DAACPs in the nervous system of Aplysia californica. Suspected DAACPs are determined by their resistance to degradation by a peptidase, Aminopeptidase M. These putative DAACPs are acid hydrolyzed into their component amino acids, which are then derivatized to enable separation of L- and D-amino acids. Next, we use Multiple Reaction Monitoring via a triple-quadrupole MS to measure L- and D-amino acid enantiomers. Finally, we confirm the DAACP assignment using standards and perform bioassays to determine the functional context.

We have already used this technique to identify a number of putative DAACPs in A. californica, including LAARLI from the procerebrin prohormone and ASSFVRIamide from the FVRIa prohormone. Funding provided by NIH through P30DA018310 and NS031609.

Keywords: Chiral, Neurochemistry, Peptides, Quadrupole MS
Application Code: Neurochemistry
Methodology Code: Mass Spectrometry
Assessment of the Liquid Sampling-Atmospheric Pressure Glow Discharge (LS-APGD) as an Ambient Desorption/ionization Source for Mass Spectrometry

Mass spectrometry has seen increased interest through miniaturized ambient desorption/ionization (ADI) sources over the last decade. Increased portability, minimal sample preparation, compatibility to most mass spectrometers, and the ability to softly generate ions are all highly regarded advantages demonstrated within the realm of ADI sources. This laboratory is investigating the use of a liquid sampling-atmospheric pressure glow discharge (LS-APGD) as an ADI source. The fundamental working principles are based on a previously published LS-APGD ionization source utilized for elemental analysis. The source is mounted onto a quadruple ion trap mass spectrometer with the plasma located ~4 mm from the sampling cone. The desorption/ionization process occurs once the sample is close enough to the microplasma (~1-2 mm), and the generated ions are carried into the sampling cone with the assistance of the sheath gas.

Preliminary results illustrated the detection of cocaine from US currency, caffeine from coffee beans and green tea leaves, and nicotine from tobacco leaves. Work presented here explores the effects of electrode geometry, including electrode positioning and angle of impedance of the ADI-LS-APGD set-up. Sampling was also investigated in terms of sample distance from the plasma as well as the sampling cone of the mass spectrometer. The understanding of these fundamental parameters will give vital information regarding the ionization process in hope of developing an efficient and versatile ADI source.

Keywords: Mass Spectrometry, Plasma, Sample Introduction, Small Samples
Application Code: General Interest
Methodology Code: Mass Spectrometry
A device is described that allows efficient synthesis of milligram quantities of organic compounds using mass spectrometric methods. Product to be efficiently collected and analyzed live is being explored. It is known that reagents in confined volumes undergo accelerated reactions. Using microdroplets and/or thin films these small volume reactions are scaled up in an apparatus that incorporates a homebuilt electrosonic spray ionization (ESSI) source and a collection surface that facilitates thin film formation. Demonstrations of carbon-carbon and carbon-nitrogen bond forming reactions and other simple chemical syntheses are shown.

The instrumentation was developed and optimized using the Claissen-Schmidt based catalyzed condensation as a model reaction. It includes a fluid pumping system, mixing systems, a modified ESSI source, gas flow controls, high voltage connections, and a collection system. The effect of experimental variables on reaction yield were studied, especially the important parameters of reagent flow, sheath gas flow, and the capillary diameter and placement. Trends from these conditions allow the user to adjust settings as needed/desired to control product yield, time, maximum gas pressure, and other factors. Coupling more sprays in an array can increase production of synthetic material.

Other reactions studied include the Hantzsch synthesis of dihydropyridines and Girard T derivatization of ketones. Air and water sensitive reactions are currently being examined. Applications can span from structure activity relation studies (SARS) in drug discovery to teaching labs to biologically relevant reactions. Extension of the capabilities to allow on-line MS analysis are also described.

Keywords: Analysis, Electrospray, Mass Spectrometry, Process Analytical Chemistry
Application Code: General Interest
Methodology Code: Mass Spectrometry
Much progress has been made in the miniaturization of ion trap mass spectrometers but the development of small, lightweight, and power efficient systems remains challenging. Reducing the pumping requirements for these systems will eliminate much of their size, weight, and power consumption. Towards this goal, our lab has demonstrated operation of small (< 1 mm), quadrupolar ion traps at pressures greater than 1 Torr, i.e., high pressure mass spectrometry (HPMS). Less progress has been made in the area of high pressure ion detection. Conventional scale mass spectrometers almost exclusively use some form of cascade electron multiplier achieving current gains up to $10^7$. Unfortunately, operation of such detectors at high pressures is limited to ~10 mTorr for even the most robust electron multipliers, far below what is needed for HPMS. A simple Faraday cup and charge sensitive amplifier scheme is attractive in that it is inherently pressure tolerant up to and exceeding atmospheric pressure and operates without the need for high voltages. We have developed and tested several Faraday cup detection systems with very high charge sensitivity. Operation of these systems in conjunction with microscale ion traps at background gas pressures up to 1 Torr in both helium and air will be presented along with comparisons to a standard electron multiplier detector.
For many years, the appearance of M+2 peaks in the mass spectra of anthraquinones and other quinone-containing compounds has been a confounding problem. Although progress on the probable experimental origin of this phenomenon was gained many years ago with the analyses of EI-generated radical cations, awareness of this progress seemed to have been overlooked when laser-based mass spectrometric analyses became in vogue. One of the intentions of this work was to marry the information gained from both types of analyses with the powerful utility of tandem mass spectrometry.

The peculiar ionization of anthraquinones, including alizarin and purpurin found in artists’ colorants, is examined. For example, as compared to ESI, when alizarin (MW = 240) is ionized by LDI it exhibits a dominant ion of [M+2H]++ at m/z 242 with a far greater abundance than the expected ion of [M+H]+ at m/z 241. Furthermore, when alizarin ionized by MALDI it exhibits the same [M+2H]++ ion with far greater abundance relative to [M+H]+, which may be due to the increase of protons from the acidic MALDI matrix (DHB).

For the first time, MS/MS analysis of these anomalous [M+2H]++ ions suggests a laser-induced photoreduction of one of the anthraquinone’s carbonyl groups. Daughter ions from neutral losses of either water or ammonia exhibit different relative abundances, depending upon their respective functional groups’ proximity to the reduced carbonyl. As proof of concept, the in situ detection of alizarin is demonstrated from both a painting cross section and a swatch of dyed silk using LDI-MS/MS.

Keywords: Art/Archaeology, Laser Desorption, Paint/Coatings, Tandem Mass Spec
Application Code: Art/Archaeology
Methodology Code: Mass Spectrometry
This work demonstrates the development and application of a spun nanoscale carbon material that was used to construct a microelectrode for the rapid detection of neurotransmitter dynamics in living tissue. Multi-walled carbon nanotubes were synthesized via chemical vapor deposition and the individual nanotubes were spun into a continuous yarn. The diameter of the finished yarn was found to be dependent on the diameter of the individual carbon nanotubes used and the twisting angle applied to the yarn. Thus, the size of the finished carbon nanotube yarn can be tailored to meet spatial constraints of an experiment adding a new scalable dimension to microelectrode fabrication. The finished yarn was glass insulated and polished to fabricate disk microelectrodes. The electrochemical performance of the carbon nanotube yarn electrodes was characterized using fast-scan cyclic voltammetry and the results demonstrate that the distinct structural and electronic properties of the nanotubes result in improved selectivity and sensitivity, as well as faster apparent electron transfer kinetics when compared to the conventional carbon-fiber microelectrodes. The disk electrodes were sufficiently sensitive for use in brain tissue, and this was demonstrated by detecting rapid concentration fluctuations of dopamine in rat striatum in response to electrical stimulation. This work lays the foundation for an improved microelectrode technology poised to expand the scope of neurochemical measurements.

Keywords: Biosensors, Microelectrode, Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Microdialysis sampling in the brain is widely used in the field of neurochemistry to understand brain function, neurological disorders and more recently, to monitor traumatic brain-injured intensive care patients. However, probe implantation causes a penetration injury that alters the surrounding tissue from its normal state, causing ischemia and gliosis. More specifically, voltammetry next to probes proves that dopaminergic activity in the striatum is disrupted after acute probe implantation. Retrodialysis of dexamethasone, an anti-inflammatory drug, had profound effects on electrically evoked dopamine release near implanted probes and tissue health surrounding the probe track four hours after implantation. These results illustrate the ability to mitigate the probe penetration injury with the use of pharmacological agents. To investigate the long-term effect of pharmacological agents on dopamine regulation near microdialysis probes, carbon fiber microelectrodes were coupled with fast scan cyclic voltammetry to monitor evoked dopamine release next to and 1 mm away from probes implanted for 24 hours. Probes were perfused with artificial cerebral spinal fluid with and without dexamethasone throughout the entire experiment. Once baseline evoked dopamine signals were obtained, nomifensine, a competitive dopamine reuptake inhibitor was administered. By acutely protecting the tissue surrounding microdialysis probes with dexamethasone, chronic mitigation of the penetration injury is possible.

Keywords: Electrochemistry, Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
The waveforms employed in fast-scan cyclic voltammetry (FSCV) measurements are often manipulated to enhance sensor characteristics such as selectivity, sensitivity, and temporal resolution. Most work has focused on optimizing FSCV parameters for in vivo detection of dopamine at carbon fiber microelectrodes. Initial dopamine studies used a triangular waveform scanning between -0.4 V and 1.0 V until it was found that extending the waveform to more anodic limits (1.3 V) increased sensitivity. However, this effect is mediated by increased dopamine adsorption through overoxidation of the carbon surface, which diminishes the time response of the electrode. It is important to understand how this limitation translates to in vivo measurements in order to interpret the role of sub-second dopamine changes in behavior.

Here we investigate the effect of the 1.0 V and 1.3 V waveforms on results obtained during intracranial self-stimulation (ICSS). ICSS is a common behavioral technique in which a rat is taught to press a lever for a rewarding electrical stimulation to its brain. Using the 1.0 V waveform, it was demonstrated that dopamine release to the lever press diminishes as the animal learns that it delivers the stimulation. These results importantly dissociated dopamine from the rewarding aspects of the lever press. To assess whether this response is evident with slower temporal dynamics, measurements have been repeated on the 1.3V waveform. In addition the 1.0 V waveform was applied at a higher scan rate (2400 V/s versus 400 V/s) to consider other means of increasing sensitivity without enhancing adsorption kinetics.

**Keywords:** Electrochemistry, Microelectrode, Neurochemistry, Voltammetry

**Application Code:** Neurochemistry

**Methodology Code:** Electrochemistry
Microfabrication offers unique advantages to the design and creation of biosensors. Standard lithographic techniques can pattern sensor designs of precise and reproducible dimensions in large batches. We have used these advantages to fabricate arrays of pyrolyzed photoresist films (PPF). These PPF biochemical sensors have more flexibility than conventionally utilized sensors such as CFMs. CFMs have been successful for selective in vivo detection of neurotransmitters, such as DA, on short time scales using fast scan cyclic voltammetry (FSCV). However, FSCV at CFMs is a differential technique unable to easily monitor the background concentrations of analytes like DA and H+ (pH). PPF arrays surpass this limitation by closely spacing two electrodes within the diffusion distance of one another to function as a collector/generator. The PPF arrays could then be used to monitor in vivo dynamics of these analytes with a post-calibration.

Keywords: Electrochemistry, Microelectrode, Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Sensors
Neurochemistry: New Approaches to Better Information from Measurements

MS Investigation of Neuropeptide Distribution and Expression Pattern Changes upon Exposure to Nanoparticles in Decapod Crustacean

With the widespread use of nano-products in our daily life, many health concerns have been raised due to exposure to nanoparticles. However, detailed molecular mechanisms are not well understood. Given the extensive regulatory role of neuropeptides in various physiological processes, we seek to measure the expression level and localization changes of neuropeptides upon exposure to Ag nanoparticles in crustacean. Herein we will implement a multi-faceted strategy based on mass spectrometry to qualitatively and quantitatively analyze the chemical messengers involved under nanotoxicity stress in the nervous system. By mapping the spatial distribution of various neuropeptide families and assessing their relative abundance levels in several major neuroendocrine organs, peptidergic regulation of nanotoxicity can be probed with unprecedented detail. In this study, different amounts of Ag nanoparticles were injected into blue crab [i]Callinectes sapidus[/i] at their leg joint while the control animal was injected with crab saline. Neuropeptides were extracted from dissected sinus gland (SG) and pericardial organ (PO). The relative expression level changes of neuropeptides observed after exposure to Ag nanoparticles were characterized by isotopic dimethyl labeling. Neuropeptides from the same family exhibited similar trends under each exposure level while different families display distinct patterns of changes at different exposure conditions. MS imaging study on PO using direct tissue analysis showed good correlation to the relative quantitation results. Under 100 [micro]L injection of Ag nanoparticles, neuropeptides from AST-B and orcokinin families showed up regulation in both relative quantitation and MSI study, while RYamides were down regulated as observed using both MS approaches.

Abstract Text

Keywords: Bioanalytical, Mass Spectrometry, Neurochemistry, Quantitative

Application Code: Neurochemistry

Methodology Code: Mass Spectrometry
Malignant gliomas account for approximately 70% of new malignant primary brain tumor diagnoses each year in the United States. Moreover, gliomas are associated with high morbidity and mortality, irrespective of treatment. The current standard therapy for newly diagnosed malignant gliomas involves surgical resection when feasible, radiotherapy, and chemotherapy. However, despite this therapy tumor recurrence still remains significant. Convection enhanced delivery (CED) is another approach used, although somewhat successful it still has serious drawbacks. This pressure-driven infusion technique lacks control over the directionality of flow from the probe tip. In CED the infusate travels away from the distal tip and up along the outside walls of the probe. It is impossible to infuse drugs into tissue near permeable structures such as ventricles, sulci, and cortical surfaces. The long term goal of our research is drug delivery into brain tissue using electroosmotic flow.

In particular, electrokinetic transport was analyzed in a series of poly (acrylamide-co-acrylic acid) hydrogels. A variety of conditions including applied electrical current and infusion time properties of the solute such as molecular weight and electrophoretic mobility were examined for optimization of delivery. Control of the directional transport of molecules was also demonstrated. Finally electrokinetic infusions were conducted in vivo in the adult rat brain. This work provides a framework for understanding electrokinetic transport and its potential uses in short-distance ejections, relevant to capillary iontophoresis, and long-distance infusions, for the clinical management of malignant brain tumors.
Fast-scan cyclic voltammetry (FSCV) is often used for *in vivo* detection and quantification of electrochemically active molecules such as catecholamine neurotransmitters. The rapid scan rates used in FSCV lead to large background currents from the charging of the double layer at the electrode surface. This background current is relatively stable over the time course of typical recordings. This allows for subtraction of the background current to obtain cyclic voltammograms (CVs) resulting from faradaic reactions. Thus, in FSCV the CV shape is sensitive not only to direct electron exchange, but also to changes in electrode surface chemistry which alter the double layer. Ionic adsorption and desorption on the electrode surface are examples of alterations to the double layer which create a unique CV for the adsorbed species after background subtraction. *In vivo* changes in oxygen and monoamines can be monitored simultaneously with an expanded potential window (+0.8 V to -1.4 V), which exhibits increased sensitivity to ionic adsorption. Adsorptive effects can interfere with currents produced from electron exchange and thus signal identification *in vivo*. To determine how ionic species affect the non-faradaic charging current at carbon-fiber microelectrodes, we characterized the adsorption of Ca$^{2+}$ at the carbon-fiber electrode surface *in vitro*. Variations on applied waveforms were tested to manipulate the sensitivity of the electrodes to Ca$^{2+}$. Finally, the *in vitro* CV’s are compared to naturally occurring ionic fluctuations recorded in anesthetized animals.
In today’s laboratory environment, new technology is one of the key drivers for improving overall efficiency in the workflow. With the introduction of UHPLC with process sample management, chromatographic analysis speed, resolution, and sensitivity are a reality in a fully automated system that gives the laboratory a quantum leap in obtaining process information. This technological advancement allows for significant improvements in laboratory workflow through richer information in reduced time. As a result of these capabilities, combined with full system automation of sampling and dilution, variability is reduced. The system configurability also allows for significant flexibility that further enhances the laboratory's capabilities from development through transfer directly to manufacturing.

Process reaction optimization is demonstrated through baseline characterization and targeted parameter adjustments and measurements. As the data is processed and understood, additional parameters can be added and evaluated for a more complete understanding of the process and the conditions that affect the outcome of the final product. Ideally suited for the task, the UHPLC technology provides faster information, resolution of closely related compounds, and detection of very low level impurities or contaminants in the presence of the target compound. The chromatographic data provided from these experiments gives the information and understanding of those key areas and decision points that are most critical and important about the process. The combination of all these capabilities provide reliable quantitative answers which other techniques cannot.

**Keywords:** Automation, HPLC, On-line, Process Control

**Application Code:** Process Analytical Chemistry

**Methodology Code:** Process Analytical Techniques
On-line analysis of pharmaceutical processes with HPLC is often necessary for reaction control, enabling the precision and sensitivity required to provide impurity control information. On-line HPLC analysis provides this information for chemical reaction optimization in research and development laboratories as well as early control points in a manufacturing environment. Fast dilution, precise sample preparation, and minimum carryover are required for successful on-line analysis. This presentation describes a commercially available on-line HPLC analysis system; as well as an in-house, custom built sample dilution system integrated with a conventional HPLC. Both instruments were used to monitor a Pd-catalyzed hydrogenation reaction. The merits of each sample preparation method and analysis will be discussed.
Polypropylene manufacturing refers mainly to isotactic PP but in the production process, an amorphous low tacticity fraction can be produced as well. In the initial stages of PP development, Natta and other scientists had to depend on extraction or solubility methods to separate PP according to stereoregularity. The atactic fraction was an unwanted amorphous material which needed to be controlled in the production process. Later on, the incorporation of ethylene to PP, which reduces the crystallizability of the resins, was of significant importance in today's very popular HIPP (High Impact Polypropylene). Traditionally, the Soluble fraction has been measured manually, using wet chemistry gravimetric methods according to ISO 6427 / ASTM D5492 standards, and using Xylene as solvent. These procedures have become a need for the PP producers, but require large amounts of solvent to be handled manually at high temperature, with safety implications given the flammability of Xylene. Furthermore, the results do not always deliver the expected precision. In response to these issues, a simpler approach has been developed, automating all the analytical process from sample preparation up to final results, thus, offering an intrinsically safer operation with no solvent handling and furthermore, using TCB or oDCB as solvents, with lower flammability risk. Thanks to the use of an IR detector capable of measuring accurately the concentration, the analyst can even skip the weighing step and get additional information about ethylene content. A capillary viscometer can also be incorporated in the system without adding significant complexity and providing IV results of the whole sample and both fractions. This new approach is not only intended to be an improved safety procedure, but it is also fully automated, delivers results with outstanding precision when measuring the Soluble fraction, and provides additional and important information of the sample microstructure.
Integration of online reaction monitoring into continuous processes has been recently gaining attention with the popularity of flow chemistry beginning to take hold. Integration of UPLC offers unique advantages in the control and understanding of continuous processes. Described here will be a process research perspective on developing a monitoring process for continuous multistep synthesis in a plug flow reactor. A two-step triazole synthesis strategy, involving explosive and toxic azides along with isolation will be described along with the integration of low level monitoring of potentially genotoxic impurities. This set of experiments was monitored by an online UHPLC system that allowed for the high speed qualitative and quantitative evaluation of the reaction conditions. This data was then incorporated into a response surface model to allow for understanding of a safe window of operations to produce material of high quality.

Keywords: HPLC, Lab-on-a-Chip/Microfluidics, On-line, Process Monitoring
Application Code: Process Analytical Chemistry
Methodology Code: Process Analytical Techniques
In-solution tryptic digestion of proteins is the typical method to obtain peptides for mass spectrometry (MS) analysis. However, to avoid autoproteolysis, these digestions use low trypsin concentrations and long digestion times. Covalent immobilization of trypsin in a porous membrane can overcome autoproteolysis and allow rapid digestion with a high local trypsin concentration. The immobilization procedure employs trypsin coupling to the activated –COOH groups of poly(acrylic acid) adsorbed in the membrane. This immobilization reduces self-digestion and yields a local enzyme density of ~35 mg/mL. The covalent linkage is stable from pH 3 to pH 9 and tolerates 1M NaCl and 6M urea, which are often required for protein solubilization. N-Benzoyl-L-arginine ethyl ester is used as substrate to test the enzymatic activity and gives ~50-fold increase in maximum velocity compared to in-solution digestion. The thin membrane reactor (110 micrometer) affords fine control over the proteolysis time to vary the lengths of peptides to optimize protein identification. The reactors can digest proteins such as apomyoglobin and bovine serum albumin in residence times as short as a few milliseconds. Future work will study whether the locations of labile proteolytic sites and the extent of proteolysis correlate with protein structure.

Keywords: Immobilization, Mass Spectrometry, Membrane, Sample Preparation
Application Code: Bioanalytical
Methodology Code: Sampling and Sample Preparation
Thin-film solid-phase microextraction (SPME) was used as sample preparation technique for the determination of cocaine and methadone in urine samples by direct analysis in real time (DART). Thin-film SPME provides efficient sample extraction and clean-up, avoiding contamination of the DART source by salt residues from the urine samples. Detectability of cocaine and methadone was also improved by using thin-film SPME: signal-to-blank ratios of 5 and 13 for cocaine and methadone respectively were obtained in the analysis of 0.5 ng/ml in urine. Extraction time was established in 10 minutes, which provides relatively short analysis time and high throughput when combined with a 96-well shaker and coupled with DART technique.
An Automated Approach for Solid Phase Extraction Methods Development for the Research Laboratory

Developing a solid phase extraction (SPE) method to extract unique or novel analytes or compounds from a sample matrix can be challenging for researchers and scientists. Different sorbents may need to be tested to determine the best type for recovery of the analytes of interest and removal of interferences. Elution solvents may be chosen on an experimental basis to determine which will give the most complete elution while using the smallest volume. The number of permutations to be executed and amount of data for evaluation can get very large. If new methods are developed or existing methods need to be adjusted for new compounds on a regular basis, the task can quickly become tedious. Automation can play a key role in helping researchers develop new SPE methods. An automated process developed for a typical method and development scenario will be described. The ability to handle different solvents and reagents, fractionate the sample and use automation to control the experimental design will be discussed.

Keywords: Automation, Extraction, Method Development, Solid Phase Extraction
Application Code: Drug Discovery
Methodology Code: Sampling and Sample Preparation
Dispersive liquid liquid microextraction (DLLME) is a popular sample preparation technique which is gaining increasing attention in analytical chemistry. This technique effectively preconcentrates analytes by dispersing a micro-volume amount of an extraction solvent into an aqueous sample solution with the assistance of a hydrophilic disperser solvent. The hydrophobic extraction solvent is then separated from the aqueous sample solution, typically by centrifugation, followed by introduction into a chromatographic system for analysis. Since the phase separation step is often time consuming and is only limited to centrifugation, the development of alternative extraction solvents and phase separation techniques in DLLME are necessary. Ionic liquids (ILs) have generated significant interest as extraction solvents in DLLME due to their low volatility and high analyte selectivity. This is due to the fact that varying the cation and anion combination can alter their physico-chemical properties. The use of magnetic ionic liquids (MILs) as highly selective extraction solvents in DLLME will be discussed. MILs are an interesting subclass of ILs which possess magnetic susceptibility originating from either the cation or the anion. Due to their magnetic susceptibility, MILs are highly applicable as extraction solvents in DLLME. The recovery of the MIL from the sample solution is faster than the traditional centrifugation step of DLLME due to the utilization of an applied magnetic field that can be introduced to the extraction system. The analytical performance and applicability of the MIL-DLLME technique for the extraction and preconcentration of nucleic acids will be discussed.

Abstract Text

Dispersive liquid liquid microextraction (DLLME) is a popular sample preparation technique which is gaining increasing attention in analytical chemistry. This technique effectively preconcentrates analytes by dispersing a micro-volume amount of an extraction solvent into an aqueous sample solution with the assistance of a hydrophilic disperser solvent. The hydrophobic extraction solvent is then separated from the aqueous sample solution, typically by centrifugation, followed by introduction into a chromatographic system for analysis. Since the phase separation step is often time consuming and is only limited to centrifugation, the development of alternative extraction solvents and phase separation techniques in DLLME are necessary. Ionic liquids (ILs) have generated significant interest as extraction solvents in DLLME due to their low volatility and high analyte selectivity. This is due to the fact that varying the cation and anion combination can alter their physico-chemical properties. The use of magnetic ionic liquids (MILs) as highly selective extraction solvents in DLLME will be discussed. MILs are an interesting subclass of ILs which possess magnetic susceptibility originating from either the cation or the anion. Due to their magnetic susceptibility, MILs are highly applicable as extraction solvents in DLLME. The recovery of the MIL from the sample solution is faster than the traditional centrifugation step of DLLME due to the utilization of an applied magnetic field that can be introduced to the extraction system. The analytical performance and applicability of the MIL-DLLME technique for the extraction and preconcentration of nucleic acids will be discussed.

Keywords: Environmental/Biological Samples, Extraction, Sample Preparation, Separation Sciences
Application Code: Bioanalytical
Methodology Code: Sampling and Sample Preparation
Sampling/Sample Preparation: Biological Applications

A Simplified Load-Wash-Elute Solid Phase Extraction Procedure for the Reversed Phase Micro Elution Plate

Solid-phase extraction (SPE) is a sample clean-up technique that can be used prior to the analysis of drugs in biological matrices such as plasma or urine. Drugs and/or their metabolites are isolated from endogenous matrix components that interfere with the precise and accurate quantitation of the target analytes. There is an increasing desire to work with smaller sample amounts (less than 100µL) for both humane and monetary reasons. We are able to work with a micro elution plate designed specifically to handle µL-volumes while still maintaining the goals of the SPE technique: clean extracts with high analyte recoveries. This plate format produces concentrated extracts in 50 µL elution volumes that can be directly injected into your LC/MS/MS, eliminating the need for the time-consuming evaporation and reconstitution steps.

In this study, a simplified Load-Wash-Elute procedure, eliminating the conditioning and equilibration steps, was evaluated using the reversed phase micro elution plate. To demonstrate the efficacy of this approach, typical samples used in drug discovery and development were analyzed. Therapeutics such as antidepressant, antiretroviral and non-steroidal anti-inflammatory drugs were used to evaluate this simplified procedure in plasma and urine. Long-term method robustness and batch-to-batch consistency of the product were demonstrated by the use of sorbent lots manufactured between 2005 and 2013. For most applications, the simplified Load-Wash-Elute procedure gives the same high analyte recoveries, low variability, and reduced matrix effects as the standard SPE procedure while reducing processing times by one third.

Keywords: Bioanalytical, Liquid Chromatography, Mass Spectrometry, Solid Phase Extraction
Application Code: Bioanalytical
Methodology Code: Sampling and Sample Preparation
Electrospinning is a highly feasible way to produce large amounts of nanofibers with diverse types of polymeric materials. The obtained nanofibers have diameters around 10s microns and large aspect ratios. Their high surface area-to-volume ratio makes it very suitable for sample pre-concentration in biological analysis. Herein, we prepared the ZrO2 electrospun nanofibers for extraction of phosphorylated peptides and proteins. Spinning and solution conditions were optimized to get fibers with average diameters around 100-200 nm but up to several micron long. High selectivity and extraction efficiency were obtained when using the fibers to isolate phosphorylated peptides, such as those present in the digest of alpha- or beta-casein, from a high background of non-phosphorylated peptides, like those in the BSA digest. The peptides were then eluted and analyzed by MALDI-TOF-MS. Additionally, we proved that the fibers were suitable to extract phosphorylated proteins at pH around 2 at good efficiency. Interestingly, adjusting the pH to 4, 6, and 8 could help with fractionating acidic, neutral, and basic proteins. The collected proteins were directly digested on-fiber and analyzed by nanoLC-MS/MS. Our study demonstrated that the ZrO2 electrospinning nanofibers were easy to prepare at low expense; and were highly effective in purification of phosphorylated peptides and in fractionation of phosphorylated proteins and proteins with distinct pI values. They will be useful in reducing sample complexity for study of protein phosphorylation during signal transduction inside cells.

Keywords: Extraction, Mass Spectrometry, Proteomics, Sample Preparation
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Sampling and Sample Preparation
Abstract Text

This study presents the evaluation of new prototypes of extended tip needle trap devices (NTD) and their performances for the in-vivo sampling of pine tree bioemission and active sampling of indoor air. The new prototypes of NT were provided by SGE Analytical Science (Victoria, Australia). The geometry of NT produced by SGE were constructed with a side hole above the sorbent and an extended tip which fits inside the restriction of the narrow neck liner to increase the desorption efficiency. For evaluation of extraction capability of SGE NTs packed with polydivinylbenzene (DVB), a series of extraction from the gas-generator chamber were carried out at 2, 5 and 10 ml/min, aspirating a volume of 20 ml with a syringe pump. The ANOVA test for all the compounds showed that at a 95% level of confidence there are not statistical differences among the 9 tested NTs. The NT is an exhaustive, solvent less and one-step sample preparation technique that can be easily calibrated. Also, it has great potential as a screening tool whenever fast analysis is needed. All features make NT suitable for field analysis. SGE NTs were successfully used for the on-site monitoring of $\Delta$-pinene, $\Delta$-pinene and limonene from pine trees. A similar concentration trend was observed in the concentration profiles obtained with different NTs along the same sampling day. Also very good performances were obtained using the NTs for indoor air analysis. Indoor air was analysed in a chemistry laboratory at the University of Waterloo. Several samples were collected in the span of a workday (8 h) to determine variations in the air contamination profile within this time limit. Active sampling through a NTD was carried out every hour to observe the intra-day variations.

Keywords: Air, Analysis, GC-MS, Volatile Organic Compounds

Application Code: Environmental

Methodology Code: Sampling and Sample Preparation
The use of alternate sampling to blood and plasma for establishing exposure to drugs has become a significant direction in clinical and forensic toxicology. The choice of saliva matrix versus blood or plasma should be addressed seriously, given the well recognized fact that saliva offers a fast and non-invasive sampling.

In this study microextraction by packed sorbent (MEPS) was used to collect exact sample volume and for sample preparation and lidocaine used as model substance. Pre-dose saliva sample (blank sample) was taken before 500 mg of Xylocaine 5% (25 mg lidocaine) were applied topically on the skin. Saliva samples were collected at 1, 1.5, 2, 3, 4 and 6 hours. A calibration curve in saliva ranged from 5.0 ng/mL to 1000 ng/mL and one zero concentration was prepared. External standard method was used. The method LQO was set to 5 ng/mL with CV of 5%.

In present study the MEPS method can handle smaller sample volumes (10-100µL). This is mainly an advantage for saliva samples from children. Moreover, MEPS is more suitable for automation and the packed syringe can be used for more than 50 times making it cost-effective.

The present study showed that the pharmacokinetic curve of lidocaine in saliva is similar to those published with plasma samples; the only different is the concentration levels are lower in saliva than plasma. A new sampling method for saliva using MEPS technique was developed (Fig. 1). We have demonstrated the use of MEPS technique with LC-MS/MS as a tool for the screening and determination of lidocaine in saliva samples. MEPS technique provides speed and the simplicity of the sample-preparation process. Furthermore, the present method reduces the sample preparation time (less than one minute per sample). The potential savings in handling time reduced solvent use. Here we provide an easy, robust, and rapid analysis for drugs in saliva. Moreover, the present method simplifies the saliva sample collection of exact volume.

Keywords: Biological Samples, Drugs, Extraction, Mass Spectrometry
Application Code: Biomedical
Methodology Code: Sampling and Sample Preparation
The successful ultrasensitive biosensing was achieved for the detection of environmental toxic small molecules, such as antibiotics, EDCs, and residual drugs by using aptamers. Aptamers are single-stranded nucleic acids having molecular recognition properties similar to antibodies, and isolated by in vitro selection and amplification process, SELEX. Five different aptamers for small toxic molecules, such as Oxytetracyclin, Tetracyclin, Ibuprofen, Diclofenac, -estradiol were successfully screened and developed in our lab. The use of unmodified gold nanoparticle-based colorimetry allow us to detect the targets fastly and simultaneously. In addition, the smallest aptamer, 8-mer, engineered through heuristic truncation was successfully conducted and validated by using modeling, the ITC and gold nanoparticle-based colorimetric assays. The results revealed that the 8-mer can bind to the backbone structure of tetracyclines, from the tests for 4 different tetracycline derivatives. About 500-fold enhanced ultrasensitive colorimetric detection of oxytetracycline was possible down to 0.1nM.

**Keywords:** Biosensors, Environmental/Biological Samples, Nanotechnology, Pesticides

**Application Code:** Environmental

**Methodology Code:** Sensors
Detecting Toxicants with a Cell-Based Impedance Biosensor

Cell-based impedance biosensors are used for fast, simple detection of environmental toxicants to understand the cellular response to chemical agents. We have developed a biosensor that consists of rainbow trout gill cells, which respond to a wide variety of toxicants, adhered on gold interdigitated electrodes (IDEs). A novel impedance measurement method, Fourier Transform Electrochemical Impedance Spectroscopy (FT-EIS), is employed to determine the change in the cells as the result of exposure to a toxicant. FT-EIS is capable of measuring impedance over large frequency spectrums in milliseconds to provide an instantaneous view of the cellular activity as compared to conventional frequency scanning techniques which takes minutes. The large frequency range offers insight into different physiological processes of the cells to further elucidate the relationship between the changes in the cell and the change in impedance. Insight into cellular susceptibilities to toxicants will be explored by comparing the impedance measurements of confluent monolayer of cells to single cells which may be exploited to design improved biosensors for environmental toxicant detection.

Abstract Text

Keywords: Biosensors, Environmental/Water, Microelectrode, Toxicology
Application Code: Environmental
Methodology Code: Sensors
Optical recognition-based chemical sensors show tremendous potential for field technicians and emergency responders, owing to the portability and low cost of such devices. Polydiacetylene (PDA)-embedded electrospun nanofibers are particularly promising as they support both colorimetric and fluorescence detection, and possess many advantages, such as high surface area, simple construction, low cost, and facile functionalization. In this work, we demonstrate new strategies for optical sensing with electrospun nanofibers that are embedded with various PDAs. The solvent-dependent fluorescent transition properties of nanofibers prepared with 10,12-Pentacosadiynoic acid (PCDA), 5,7-eicosadiynoic acid (ECDA), and N-(2-(2-(2-aminoethoxy)ethoxy)ethyl)pentacosa-10,12-diynamide (PCDA-EDEA) generated a fluorescent pattern that successfully distinguished four common organic solvents. The colorimetric and fluorescent transitions of biotin-avidin interactions by embedding biotinylated PCDA monomers into silica-reinforced nanofiber mats to detect biomolecules were also investigated. Finally, a PDA-based nanofiber sensor array consisting of PCDA, ECDA, and 10,12-docosadiyndioic acid (DCDA) is reported for the determination and identification of organic amine vapors using colorimetry and principal component analysis (PCA). The combination of PCA and use of data from two different analyte concentration ranges (ppm and ppth) led to successful analysis of all 8 amines.

Keywords: Nanotechnology, Pattern Recognition, Sensors
Application Code: Environmental
Methodology Code: Sensors
There is an urgent need to detect biodiesel at low and high concentrations in diesel. The current techniques for the detection of biodiesel, which generally consists of fatty acid methyl esters (FAME), rely on GC, HPLC, and IR. We have found that solvatochromism can be used to detect FAME/biodiesel in diesel. Based on this discovery, we have developed, for example, a simple, optical sensor containing the dye, Nile Blue chloride, in a thin film for quick and direct biodiesel detection. The solvatochromatic properties of the dye lead to a color change from blue in diesel to pink in the presence of FAME. This highly sensitive, disposable sensor detects 0.5-200,000 ppm (20% v/v) FAME/biodiesel in diesel. The sensor response to 0.5–30 ppm FAME in diesel (20 mL) is less than 30 min. At concentrations higher than 1,000 ppm, the sensor response time is less than 5 min. This novel sensor may be used for both low, ppm-level detection of FAME in diesel and high-level measurement of biodiesel at, e.g., B20, in biodiesel-diesel blend.
Capillary Zone Electrophoresis and ElectrosprayIonization-tandem Mass Spectrometry for Top-Down Intact Secreted Protein Characterization

Top-down proteomics directly measures the mass-to-charge ratio of intact proteins by mass spectrometry. It provides advantages in protein sequence coverage as well as identification and characterization of protein isomers and post-translational modifications. However, due to limited mass spectrometry resolution and spectra complexity of intact proteins, top-down approach is usually applied in single purified protein analysis. To simplify spectra, liquid chromatography is often used in protein separation, but the separation is not as efficient as for peptides. In this work, we employed online CZE separation with sheath-flow ESI interface and high-resolution tandem mass spectrometry (Q-Exactive) to analyze secreted protein of Mycobacterium marinum. Secreted protein was obtained directly from secretome by acetone precipitation. The protein precipitate was then dissolved in 70% acetic acid and separated in a LPA coated capillary followed by mass analysis. Each separation finished within 50min and the separation window was about 35min. The resulted tandem spectra were first decharged and deisotoped by MS-Deconv. After database searching with MS-Align+, 22 proteins were identified from a single run with E-values less than 1e-4. Post translational modifications including N-terminal methionine excision and acetylation, signal peptide removal and various internal modifications could be identified. Fragmentation with the optimized normalized collision energy of about 30% provided confident identification of intact proteins. These results demonstrated that CZE-ESI-MS/MS is a promising platform for top-down complex intact protein mixture characterization. This work was funded by the National Institutes of Health (R01GM096767) and Eli Lilly Company through the Lilly Research Award Program.

Keywords: Capillary Electrophoresis, Mass Spectrometry, Proteomics
Application Code: Genomics, Proteomics and Other ‘Omics
Methodology Code: Capillary Electrophoresis
Huntington’s disease (HD) is a fatal, genetic, neurodegenerative movement disorder characterized by loss of motor control and also by mental/emotional problems, such as depression. Depression, in particular, is highly prevalent among HD patients and represents a significant risk factor in committing suicide. Given the well-established association between depression and serotonin release and reuptake, fast-scan cyclic voltammetry at carbon-fiber microelectrodes was used to measure serotonin release in coronal brain slices from R6/2 mice and age-matched wild type controls. Electrical stimulation was used to evoke serotonin, and a waveform of -0.7 V to +1.3 V at 400 V/s was applied at the working electrode. It was found that serotonin release in R6/2 mice is significantly impaired in 12 week-old mice compared to age-match wild-type control mice.

Keywords: Electrochemistry, Neurochemistry, Quantitative, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Dorsal root ganglia (DRG) are the part of the peripheral nervous system. They consist of a variety of cells including neurons which are responsible for the collection and partial processing of sensory information from the periphery to the brain. Peptides secreted from DRG cells are thought to play a role in cell-to-cell signaling. In this work we present the model and strategy for identifying the peptide components of regulated secretion from murine DRG cells. DRG cells were cultured in a glass-polydimethylsiloxane (PDMS) microfluidic device. The device allowed temporal stimulation of the cells through the application of elevated extracellular potassium (60 mM KCl). Releasates were collected before and after the cell stimulation at different time points. Released peptides were enriched and partially purified with C18 ZipTips pipette tips, and subjected to matrix-assisted laser desorption/ionization mass spectrometry. The comparative analysis of sets of peptides detected in releasates collected before and after stimulation uncovers a subset of putatively functionally important features. These features were matched to peptides from DRG peptides extracts, which were identified by liquid chromatography mass spectrometry. We also observe the differences between profiles of released peptides collected at different time periods after stimulation, this opens a way for studying the temporal dynamics of peptide release from DRG cells.

Acknowledgment: P30 DA 081310

Keywords: Bioanalytical, Lab-on-a-Chip/Microfluidics, Mass Spectrometry, Peptides
Application Code: Neurochemistry
Methodology Code: Mass Spectrometry
In vivo fast-scan cyclic voltammetry (FSCV) in conjunction with carbon fiber microelectrodes enables the real-time, in vivo detection of dopamine, a significant neurotransmitter in the mammalian central nervous system. Recent applications of this approach have revealed that key dopamine terminal fields in the brain are organized into patchworks of distinct kinetic dopamine that exhibit different rates of dopamine release and reuptake that respond uniquely to various drugs. Nicotine, an active and addictive component of tobacco, is thought to act on brain dopamine systems but no studies of nicotine's potential domain-dependent actions on dopamine have yet been reported. We implanted carbon fiber microelectrodes (7 μm in diameter and 200 μm in length) into the striatum of isoflurane-anesthetized rats and performed FSCV (400 V/s, 10 Hz) during electrical stimulation of dopamine axons (60 Hz, 250 μA). The focus of attention in this study is the slow dopamine domains, since these have been less widely studied compared to the fast domains. Nicotine (0.1 mg/kg i.v.) has highly variable effects on evoked dopamine in slow domains, sometimes increasing, sometimes decreasing, and sometimes not affecting the response amplitude. These finding suggest a complex and diverse pattern of cholinergic regulation of dopamine's slow kinetic domains. (This work was supported by grants from NIH: MH 075989 and NS 081744)

**Keywords:** Electrochemistry, Microelectrode, Neurochemistry, Voltammetry

**Application Code:** Neurochemistry

**Methodology Code:** Electrochemistry
Electrophoretically Mediated Microanalysis (EMMA) is applied to the measurement of creatinine through the Jaffe reaction to efficiently perform a clinically significant reaction on the nano-liter scale. Performing this reaction within a capillary tube requires only small volumes of the reacting solutions and can be completed more rapidly than with the conventional approach. Correct overlap of the reactant plugs and electrophoretic stacking of the in-line product is critical to the sensitivity of the assay. This work involves monitoring reaction yield and stacking efficiency as overlap and local reaction ionic conditions are systematically changed. Artificial blood serum matrix is used to mimic real sample content. In addition to wet lab experiments, Simul, a computer simulation program, is used to visualize the dynamics of the overlap and stacking processes, allowing a detailed understanding of this complex EMMA-based reaction system. Recent results pointing to optimal conditions for the analysis in Borate buffers of various concentration and/or pH will be presented. Ultimately the careful characterization of this system may lead to a more general understanding of EMMA dynamics and allow for prediction of optimal conditions for in-line electrophoretic analyses.

Keywords: Bioanalytical, Capillary Electrophoresis, Separation Sciences
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Automated diagonal capillary electrophoresis is a two-dimensional separation method that incorporates an immobilized enzyme reactor at the distal end of the first capillary and employs identical electrophoretic separation modes in both dimensions. Components undergo a preliminary separation in the first capillary. Fractions are parked in the reactor where some components undergo transformation. The fractions are then periodically transferred to the second capillary and replaced by the next components in the sample. Components that are not modified by the reactor will have identical mobility in both dimensions and fall on the diagonal of a reconstructed two-dimensional electropherogram, while analyte that undergo modification will fall off the diagonal.

The system was used to characterize the phosphorylation status of a tryptic digest of β-casein in a background prepared from a 22-fold excess of the tryptic digest of bovine serum albumin. 120 fractions underwent automated treatment over 40-minutes; nine phosphorylated β-casein peptides that produced 20 different phosphorylation states were detected with high confidence. The system also showed good accuracy and precision for further study of extent of phosphorylation analysis.

**Abstract Text**

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**Keywords:** Bioanalytical, Capillary Electrophoresis, Proteomics, Quantitative

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

**Methodology Code:** Capillary Electrophoresis
An Organic Light-Emitting Diode (OLED) Induced Fluorescence Detection System for Use in a Compact Disk-Type Microfluidic Device

Compact disk (CD)-type microfluidic device is useful for on-site measurement since pumps and valves are unnecessary. Laser-induced fluorescence is often used as detection method for the CD-type microfluidic device. However, large-scale and expensive peripheral equipment such as a laser and a microscope are necessary, which enlarge the total size of the analytical system. In this study, a novel portable fluorescence detection system for use in conjunction with a CD-type microfluidic device was developed using an organic light-emitting diode (OLED) and a CCD.

The fluorescence detection system consisted of a laboratory made power source for OLED, an OLED device, two band pass filters, a half-ball lens, a CD-type microfluidic device and a CCD.

The developed fluorescence detection system was applied to ELISA on the CD-type microfluidic device for the simple and rapid determination of IgA in human saliva, a marker for human stress. The detection limit (S/N=3) for IgA was 35.9 ng/mL. The sensitivity of detection of this system is sufficient to permit it to be used to evaluate human stress, since human saliva contains 110-220 μg/mL of IgA. Conventional ELISA on a 96-well microtiter plate requires 150 min for the determination of IgA, whereas using this set up, it is possible to determine and quantitate IgA in ca. 20 min. The amounts of reagent and sample required for the analysis were also reduced by ca. 1/10 compared with the amounts required for a conventional 96-well microtiter plate assay.

Keywords: Detection, Fluorescence, Immunoassay
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Simultaneous separation and quantification of seven parabens methyl, ethyl, isopropyl, propyl, isobutyl, butyl, and benzyl p-hydroxybenzoate, commonly used as preservatives in cosmetic products, by MEKC with an stacking technique has been demonstrated. An effective on-line concentration strategy involving a combination of sweeping and the use of polymer solutions is a key feature of the proposed method, which successfully determined individual parabens. The analysis parameters such as injection time, pH and concentration of phosphate solution, and concentration of SDS and poly(ethylene oxide) (PEO) were examined. The optimum conditions were found to be as follows: a 15 mM phosphate solution (pH 9.5) containing 20 mM SDS for filling the capillary, and for the separation electrolytes, 0.100% PEO (8 MDa) added to the phosphate and SDS solution of the same composition as for the capillary. The entire analysis process was completed in 13 min and a 930–2200-fold enhancement factor was achieved. The LODs (S/N = 3) for this approach were in the range from 4.32 to 7.78 nM. The linear range for each paraben was between 50 nM and 5.0 μM (R2 > 0.990). The optimized method was then successfully applied to the determination of parabens in commercial cosmetic products.

Keywords: Analysis, Capillary Electrophoresis, UV-VIS Absorbance/Luminescence

Application Code: Process Analytical Chemistry
Methodology Code: Capillary Electrophoresis
Primary fatty acid amides (PFAMs), a subclass of fatty acyls, have been found in several specific tissues and biological fluids, and have been shown to exhibit a wide range of physiological effects. Endogenous PFAMs have been found in the nM range, requiring sensitive detection systems for quantitation at physiological concentrations. Mass spectrometry has been predominantly used as the optimal detection system; however, its limits of detection and quantitation fall on the periphery of biologically native PFAM concentration, thus, laser induced fluorescence (LIF) becomes an attractive detection system as it presents the ability to approach single molecule detection limits. Microfluidics enables both chemical reactions and detection to be performed on-chip at nL volumes. By utilizing droplet-based microfluidics, reaction efficiency and time are greatly improved, allowing primary fatty amines (PFAM derivatives) to be fluorescently tagged at physiological concentrations. PFAMs must be converted to their conjugate amines prior to fluorescent tagging due to the delocalization of electron density caused by the carbonyl group on the amide. Naphthalene dicarboxaldehyde (NDA) was used for fluorescent tagging reactions and subsequently tagged amine standards were serially diluted to show detection limits of five fmol/amine using reverse phase high performance liquid chromatography coupled to a fluorescence detector. The use of a microfluidic platform with LIF and single photon counting detection system has currently lowered detection limits to the low amol/droplet range. Droplet-based microfluidics acts as total analysis system enabling chemical separation, fluorescent tagging, and detection to all be performed on-line with little user interaction, thus increasing reproducibility and throughput. The optimized methodology will be used to determine endogenous concentrations of PFAMs extracted from biological samples.

Keywords: Fluorescence, HPLC, Lab-on-a-Chip/Microfluidics, Ultratrace Analysis
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Capillary zone electrophoresis (CZE) could provide rapid separations and complementary information for bottom-up proteomics compared with liquid chromatography (LC). Because of the CZE-MS interface, CZE-MS starts to have broader use in proteomics. In the normal setup, CZE-electrospray ionization-tandem mass spectrometry (ESI-MS/MS) could only provide hundreds of peptides information in each single shot. After optimization, the system employed an electrokinetically-pumped interface, a LPA-coated capillary, and stacking conditions for sample injection, which would be applied for analysis of seven fractions of E.coli protein digest. During each 50 min analysis, 100 ng fractioned digests would be loaded in, respectively. Seven fractions were collected using solid phase extraction (SPE) and analyzed by CZE-ESI-MS/MS. The 60 cm LPA-coated capillary (50 μm i.d./150 μm o.d.) here is for peptide separation, and the separation buffer is 0.1% (v/v) formic acid. In order to improve the separation performance, stacking condition is employed in this system through dissolving protein digests in 50% (v/v) acetonitrile and 0.05% (v/v) formic acid. An electrokinetic sheath-flow electrospray interface was used to couple the separation capillary with an OrbitrapVelos operating in higher-energy collisional dissociation (HCD) mode. Each CZE-ESI-MS/MS run lasted 50 minutes with 100ng fractionated digests and total MS time was 350 minutes. Over 871 protein groups and 4902 peptides were generated from 350 min analysis, which is the largest dataset for CZE-MS/MS system.

This work was supported by a grant from the National Institutes of Health (R01GM096767).

Keywords: Capillary Electrophoresis, Mass Spectrometry, Solid Phase Extraction
Application Code: Genomics, Proteomics and Other ‘Omics
Methodology Code: Capillary Electrophoresis
While multidimensional gas chromatography has proven to be an invaluable tool for the separation of volatile metabolites, there have been difficulties in conducting equivalent liquid separations for non-volatile metabolites. Two dimensional liquid chromatography (LC) can be slow and laborious, and maintaining constant pressure through both dimensions is challenging. Capillary electrophoresis (CE) offers many advantages over conventional LC, including efficiency, speed and reduced sample consumption. Until recently, the speed of electrokinetic separations has been limited by data acquisition rates of current mass spectrometers. We have coupled our CE separations to a Thermo LTQ XL mass spectrometer, with data acquisition settings optimized to collect spectra as fast as possible. We have developed a fast (<1 min) and effective one-dimensional separation protocol for a complex mixture of amino acids with a separation window of ~40 seconds and peak widths <0.5 s. Future aims include expanding high-speed separations to other classes of metabolites and developing fast, multidimensional electrokinetic separations for a complex mixture of metabolites.

Keywords: Capillary Electrophoresis, Mass Spectrometry, Metabolomics, Metabonomics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Capillary Electrophoresis
A new microfluidics chip for various imaging purposes has been designed. This passively operated device is capable of completing one-to-one reagent mixing, signal and reference droplet generation (µChopper from our previous work), incubation, and detection. The chip comprises a 50 [micro]m deep droplet generation and reagent mixing region followed by a 100 [micro]m deep incubation region. The incubation channels include 466 chambers of 470 [micro]m width, including constrictions between chambers to maintain droplet ordering around turns. Incubation times more than 12 minutes were achieved, which should be useful for fully-integrated endocrine cell secretion sampling and FRET-based hormone assays. This device has also proven useful for generation and imaging of DNA-containing droplets for isothermal amplification and analysis in picoliter volumes. In future work, it should be possible to apply this device for fully-integrated hormone sampling and quantification as well as fully-integrated digital DNA detection using isothermal amplification techniques.
Latex Nanoparticle Pseudo-Stationary Phases for Electrokinetic Chromatography: Influence of the Ionic Shell

Organic nanoparticles have recently been introduced as pseudostationary phases for electrokinetic chromatography (EKC), with a significant advantage being compatibility with mass spectrometric detection. Additionally, the synthetic approach utilized to generate these nanoparticles is highly versatile, and this presents opportunities to tune and optimize their performance. In the present study, the performance and selectivity of novel nanoparticles based on poly acrylate and sulfonic acid shells, synthesized by reversible addition–fragmentation chain transfer (RAFT) polymerization, have been studied using EKC. The behavior and performance of the new pseudo-stationary phases are characterized as a function of chemistry and structure. The thickness and ionic moiety of the latex nanoparticle shell are found to have very little effect on the EKC performance of the latex nanoparticles, even when strongly acidic sulfonic acid functional groups are incorporated into the shell. Regardless of shell chemistry, the latex nanoparticles exhibit high mobility even in low pH environments. This allows for control of analyte ionization and electroosmotic flow rate by adjusting background electrolyte pH.

The linear solvation energy relationship (LSER) model was applied to characterize the solvent properties and interactions between the nanoparticles and analytes. The solvent characteristics of the nanoparticles were found to be similar to other acrylate based co-polymers, and are not significantly altered by changes in the ionic group chemistry. The overall solvent properties of the nanoparticles imply that the hydrophobic core of the particles are highly accessible, while interactions with the ionic shell are weak or non-existent. These fundamental studies of nanoparticle performance and selectivity should lead to better applicability and informed design of new nanoparticle pseudo-stationary phases for EKC.

**Keywords:** Capillary Electrophoresis, Chromatography, Materials Science, Separation Sciences

**Application Code:** General Interest

**Methodology Code:** Capillary Electrophoresis
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. A method has been developed for analyzing secretion and uptake of amino acids in adipocytes. Currently there are no adequate methods to measure these dynamics. Human pre-adipocytes have been cultured and differentiated into 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-benzoxadiazole (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 almost real time analysis of cellular secretions. Separation conditions have been optimized for the separation of the BCAAs. Cellular growth was monitored when cultured with various surface modifiers (poly-lysine, gelatin, and fibronectin) in order to obtain optimum growth for microdialysis measurements. 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.

**Keywords:**  Amino Acids, Capillary Electrophoresis, On-line

**Application Code:**  Bioanalytical

**Methodology Code:**  Capillary Electrophoresis
Electro-Transfer Efficiency of Various Protein Types Using an Automated a Semi-Dry Method for Western Blot Analysis

The detection of target proteins with Western blot analysis is dependent upon efficient transfer to a membrane substrate. The transferring of protein bands from gels to nitrocellulose (NC) or polyvinylidene difluoride (PVDF) to make them accessible to detection has been used in biochemistry laboratories since the mid 1970’s. Two distinct advantages of the current automated semi-dry transfer instrumentation are their speed and ease of use relative to traditional “wet” transfer. However, consideration is often not given to the possibility that transfer by these methods does not always result in complete recovery of protein on the membrane for immuno-detection. Incomplete recovery due to inefficient transfer of specific proteins can thus confound the detection of a target antigenic species by Western blotting methods. Prediction of electro-transfer efficiency for a protein with known or theoretical properties would aid in the design of an analytical method to assure detection and interpretation of results near the detection limit.

In our studies we have measured transfer efficiencies for several standard proteins to correlate with bio-molecular properties (e.g. size, charge, post-translational modification). Transfer efficiencies were determined by densitometric measurement and comparison of stained protein quantities in gels and on blots, pre and post transfer. Transfer efficiencies were found to depend on a variety of specific protein characteristics, in addition to molecular weight (MW). The scope and limitations of the automated semi-dry transfer method will be presented as well as potential application in research and quality control lab use.

Keywords: Electrophoresis, Identification, Protein
Application Code: Bioanalytical
Methodology Code: Other (Specify)
Tissue phantoms are synthetic imitations of biological tissue that mimic specific characteristics such as optical, acoustic, thermal, or mechanical properties. These properties can be adjusted in the synthetic material to simulate disease progression. Of particular interest for cancer diagnosis, the spectroscopic properties of tissue – scattering, absorbance, and fluorescence – can be modeled in a phantom by incorporating fluorescent biomolecules that are indicative of metabolic rate. These phantoms could be used to develop a highly sensitive, non-invasive optical method of cancer detection. Mesoporous silica particles functionalized with octadecylsilane – suspended in agar – were selected as a loading platform for fluorophores because these particles provide biochemically well-defined micrometer and nanometer domains due to hydrophobic trapping of loaded molecules.

Uniformity of the tissue phantom samples that are used to calibrate an optical device is critical to the development of a robust diagnostic methodology. One barrier to generating uniform phantoms is the tendency of hydrophobic particles to aggregate in agar, the aqueous suspension matrix. Current efforts to disperse particles include surfactant coating and various methods of physical dispersion. An analysis method utilizing the pair correlation function has been developed to quantify particle clustering as a means of evaluating the particle dispersal in the resulting tissue phantoms. Different particle dispersal patterns – clustered, uniform, and random – are distinguishable based on characteristic features produced in the pair correlation function. The robustness of the mathematical analysis of the confocal microscopy phantom images is established using simulated images with various dispersal patterns. This work is funded by the NIH and the University of Iowa’s Center for Biocatalysis and Bioprocessing.
Physiological buffer (acid-base) regulation is the crux for a healthy life. Almost all biological processes produce acid as a by-product that can affect the pH of blood as it flows through the body. The body maintains a normal pH of 7.4 through various homeostatic adaptations including respiration. Traumatically injured patients and those suffering from sickle cell disease (SCD) have a compromised immune system rendering these adaptations ineffective and reducing the body’s buffering capacity and ability to maintain pH. This, in most cases acts as a precursor to debilitating health conditions leading to stroke, pulmonary hypertension and fatalities. For doctors in accident and emergency wards, a prompt and accurate assessment of a patient’s physiological state can aid a quicker diagnosis. Additionally, understanding buffer capacity can help medical practitioners treating SCD patients reach a faster diagnosis and also assess the efficacy of their treatment protocols. Consequently, the present research addresses the need for a quick and simple lab-on-chip system that can assess buffer capacity of physiological samples coulometrically, circumventing the use of chemical titrants. The design is based on the characteristics of the carbonic acid-bicarbonate buffer which is responsible for 80% of the body’s buffering capacity. A local pH of 5.4 is indicative of 90% buffer exhaustion, or titration endpoint, which can easily be detected using pH indicators signalling an abrupt colour change. Using this endpoint detection, a comparison between a reference sample (known buffer capacity) and a working sample (unknown buffer capacity) can yield a difference in the pH indicator’s colour change which can electronically be quantified to give a close to accurate estimate of the sample’s residual buffer capacity. Based on the assessment, an infusion of bicarbonate ions can temporarily abate the emergency and save lives until further tests are undertaken to assess the underlying cause.

Keywords: Biosensors, Electrochemistry, Lab-on-a-Chip/Microfluidics, Titration
Table olives require a thorough process of debittering in order to achieve the organoleptic quality of olives before consumption. Several chromatographic analytical methods have been used in order to ascertain the debittering process. Capillary electrophoresis (CE) offers several advantages to the determination of the bitter responsible compound oleuropein in table olives, such as speed, reduced amount of solvents and reagents (green chemistry), small amount of sample, high efficiency and lower costs. Despite the evident advantages of CE, this technique has not been used so far for the verification of the debittering of table olives.

Our objective was to extract oleuropein from table olives and optimize the analysis via CE using a design of experiments.

Soluble biophenols, oleuropein among them, were extracted using sequential liquid partition methods with methanol:acetone and hexane from table olives as previously described (1). Finally the samples in methanol were transferred to vials. CE was run in a P/ACE System using an uncoated fused-silica capillary (50 μm ID x 375 μm OD, 50 cm length). UV detection performed at 214 nm in cationic mode with pressure injection for 7 s.

The optimization was accomplished by means of a central composite design using as response the resolution between oleuropein and its nearest peak. A preliminary experiment screened the appropriate parameters that required further optimization. The factors for the central composite design were: separation buffer pH, voltage and buffer composition. The best separation was achieved at 21ºC, voltage 10 kV, and 20 mM tetraborate in 20 mM phosphate buffer at pH 10.0.

An innovative assay with fast and ultrasensitive response for protein detection has been developed by using gold nanoparticles-silica nanorods (AuNPs-SiNRs) and lateral flow technique. The goat anti-rabbit IgG antibodies (Ab1) conjugated with AuNPs-SiNRs were captured at test line (capture antibodies) through antibody-antigen sandwich immunoreaction, using Rabbit IgG as a model protein analyte. The detection sensitivity was enhanced owing to the large surface area of SiNRs coated with numerous AuNPs, allowing increasing conjugation amount of Ab1 and intense purple black color of test line. Under the optimal conditions, the proposed method presented fast and sensitive detection of Rabbit IgG over a linear range of 0.05-2 ng/mL, and the detection limit was 0.01 ng/mL, which was 50-fold lower than that based on AuNPs as signal labels. Therefore, this AuNPs-SiNRs based lateral flow bioassay was successfully established as a rapid, cost-effective and simple method for quantitative analysis of trace amount of protein biomarkers and has a promising application for point-of-care screening in clinical diagnostics or other biomedical researches.

Keywords: Bioanalytical, Biosensors, Materials Science, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Sensors
While there are effective treatment regimens for certain types of stroke, only 2% of these treatments actually reach the appropriate patient pool due to the time sensitive nature of their administration and the poor diagnostic strategies currently available. Stroke diagnoses are hampered by a lack of acceptable biomarkers and the assays to analyze them. Recent studies have shown that there are significant changes in the activity of some genes, especially those genes found in T-cells, due to the onset of a stroke event. This change in gene expression could be an important target for stroke diagnosis. The overall goal of this research is to develop a microfluidic bioprocessor that can be used to quantitate specific mRNA transcripts in T-cells, extracted from whole blood. To accomplish this quantitation, total RNA or mRNA needs to be isolated from the subset of cells of interest, and then reverse transcribed into cDNA for eventual detection. In this work, total RNA isolation was accomplished via solid phase extraction (SPE) on a polycarbonate (PC) microfluidic chip. RNA extraction efficiencies via SPE were determined to be 57% for 5ng of TRNA processed on-chip. RNA was extracted from CD4+ T-cells isolated from blood, and successful RT-PCR of the captured RNA demonstrated a difference in expression levels for two gene markers. The use of on-chip continuous flow (CF) RT was evaluated in a UV-modified cyclic-olefin copolymer (COC) chip for different reaction times, and compared to benchtop reactions. CF-RT was accomplished and product was seen for as low as one minute reaction times.

Keywords: Biological Samples, Lab-on-a-Chip/Microfluidics
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Abstract Text
Human serum albumin (HSA) is the most abundant serum protein in blood and a common transport protein for many
drugs in the body. Information on the kinetics of the drug interactions with HSA is valuable in understanding the
pharmacokinetic behavior of such drugs and could provide data that might lead to the creation of improved assays for
these analytes in biological samples. In this study, the dissociation rate constants for verapamil, diazepam, disopyramide
and lidocaine with HSA were determined by using high-performance affinity chromatography along with affinity
microcolumns and peak profiling. The peak profiling method was used to compare the elution profiles for each drug and a
non-retained species on an HSA column and control column over a broad range of flow rates. Dissociation rate constants
in the range of 0.28 to 0.45 s⁻¹ were obtained at pH 7.4 and 25°C on a 1 cm × 2.1 mm i.d. microcolumn for the
interactions of immobilized HSA with verapamil and diazepam, giving results that had good agreement with other
methods. The same approach was used to examine the interactions between disopyramide and lidocaine with HSA, giving
dissociation rate constants that ranged from 0.28 to 0.35 s⁻¹. This method described in this study for kinetic studies is a
relatively fast approach that is not limited to these particular drugs or HSA but could be extended to other drugs and
proteins or other types of biomolecular interactions.

Keywords: Drugs, HPLC, HPLC Columns, Protein
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Affinity reagents bind to target molecules with high specificity and selectivity. Identification, tracking, capture, or altering the activity of the target can be accomplished by an antibody, peptide, or nucleic acid (aptamer) with affinity for said target. The need for affinity reagents against large numbers of proteins is prevalent and their generation is typically time and cost consumptive. We aim to circumvent the current technology’s inefficient consumption of resources through the production of an innovative instrument designed to generate aptamers with high affinity for specific protein targets in a high-throughput fashion. We present a CE instrument interfaced with a fully automated and highly efficient fraction collection instrument programmed to dispense sample effluent from the distal end of the capillary along with a precise amount of standard reagents and specific primers into a 96-well plate for aptamer amplification and detection by real-time PCR.
Analysis of Electrophoretic Separation Methods for Purity Testing of an Atypically-Reactive Recombinant Antibody

Biologically-manufactured protein reagents for use in clinical diagnostic assays require stringent purity testing to assure effective and stable performance. Analytical methods employing polyacrylamide gel (SDS-PAGE) and capillary (CSE) electrophoretic separations have been employed for decades as standard approaches to purity measurement of diagnostic biomolecules such as antibodies and antibody fragments. Purity testing for an antibody protein with these methods is typically performed on a reduced and denatured analyte that yields isolated light and heavy polypeptide chains for separation based exclusively on size. Presented here is a case where the standard reduction and denaturation sample preparation for a recombinant rabbit antibody induces a subsequent unique oligomerization reaction to produce distinct species with apparent size significantly larger than the original intact antibody molecule. The instability of the isolated heavy and light chains towards oligomerization in standard sample matrices for both SDS-PAGE and CSE methods thus challenges a true purity assessment of this atypical antibody, and would render these methods completely untranslatable to utilization in quality control testing. The oligomerization reaction was found to be induced by introduction of the reducing agent, as the denatured non-reduced full antibody molecule does not form these oligomers. Induction of the oligomerization reaction was insensitive to the type of reducing agent applied and also occurs with an expressed recombinant Fab' fragment construct of this same antibody. Results from various experiments with alternate sample preparation procedures toward optimization of a method for purity test implementation will be presented.

Abstract Text

Bioanalytical Neurochemistry, Capillary Electrophoresis, Electrophoresis, and Microfluidics

Optimization of Electrophoretic Separation Methods for Purity Testing of an Atypically-Reactive Recombinant Antibody

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Keywords: Bioanalytical
Application Code: Bioanalytical
Methodology Code: Bioanalytical

Date: Wednesday, March 05, 2014 - After
Time:
Room: - Exposition Floor, Back of Aisles 1
The olfactory tubercle (OT) receives both dopaminergic and noradrenergic innervations and plays an important role in behavior and the reward system. Although previous studies suggested that dopamine (DA) in the OT may modulate the regulations of locomotor activity, incentive salience, and drug abuse, the regulations of extracellular DA and norepinephrine (NE) have not been fully understood yet due to anatomical and technical limitations. Carbon-fiber microelectrodes (CFME) possessing high temporal and spatial resolutions allow us to study dynamic regulation of neurotransmitters in small nuclei, a few hundreds of microns in diameter. Here, we investigated characteristic features of DA and NE overflow in the subregions of the OT of anesthetized rats using fast-scan cyclic voltammetry (FSCV) following electrical stimulation of dopaminergic cell bodies and noradrenergic projection pathways. The concentration of the catecholamine evoked by the electrical stimulation was only increased by the dopamine D2 antagonist and the dopamine uptake inhibitor but not by the β adrenoceptor antagonist and the NE uptake inhibitor. These electrochemical, pharmacological, and anatomical results indicated that DA was the major catecholamine in the anterior/posterior OT.

Abstract Text
The olfactory tubercle (OT) receives both dopaminergic and noradrenergic innervations and plays an important role in behavior and the reward system. Although previous studies suggested that dopamine (DA) in the OT may modulate the regulations of locomotor activity, incentive salience, and drug abuse, the regulations of extracellular DA and norepinephrine (NE) have not been fully understood yet due to anatomical and technical limitations. Carbon-fiber microelectrodes (CFME) possessing high temporal and spatial resolutions allow us to study dynamic regulation of neurotransmitters in small nuclei, a few hundreds of microns in diameter. Here, we investigated characteristic features of DA and NE overflow in the subregions of the OT of anesthetized rats using fast-scan cyclic voltammetry (FSCV) following electrical stimulation of dopaminergic cell bodies and noradrenergic projection pathways. The concentration of the catecholamine evoked by the electrical stimulation was only increased by the dopamine D2 antagonist and the dopamine uptake inhibitor but not by the β adrenoceptor antagonist and the NE uptake inhibitor. These electrochemical, pharmacological, and anatomical results indicated that DA was the major catecholamine in the anterior/posterior OT.

Keywords: Biosensors, Electrochemistry, Neurochemistry
Application Code: Bioanalytical
Methodology Code: Electrochemistry
The most effective stroke treatments can only be given within the first few hours after a stroke has occurred, with misdiagnosis leading to life-threatening outcomes as the therapies for the hemorrhagic and ischemic stroke are very different. Current diagnostic tools like computed tomography (CT) and magnetic resonance imaging (MRI) cannot distinguish between these two types of strokes, and also cannot be implemented in the field for a diagnosis in a turn-around-time. It has been found that the gene expression of neutrophils and T-cells has been altered during stroke. We are developing a technology platform for a rapid molecular diagnosis of stroke using mRNA biomarkers in white blood cell subsets relevant to stroke diagnosis. Target cells were selectively isolated from blood using a polymeric microfluidic device consisting of four capture beds with curvilinear channels 25[$\mu$m] wide and 80 [$\mu$m] deep and modified with antibodies specific to target antigens. Different device geometries were also evaluated for the cell capture. CD4+ T-cells and neutrophils were isolated with purities greater than 90% using affinity based capture in COC-based devices. RT-PCR after the lysis of pure cells showed the expression of T-cell specific gene, TCRBC and neutrophil specific gene, FPR1. We have proposed a capable affinity based microfluidic system for isolating highly pure sub-types from a cell mixture in blood. The modification and isolation procedures demonstrated here can also be expanded to analyze other cell types.
Olive oil has become a valuable vegetable oil because of its high nutritional and health values. Olive oil consumption is progressively increasing worldwide, notwithstanding its higher price, compared with other edible oils. The beneficial features of olive oil can be damaged by fraudulent activities such as dilutions or adulterations, making imperative the certification of olive oil authenticity. DNA is less influenced by environmental and processing conditions, and PCR is a sensitive and reliable technique, therefore the aim of this work was to determine the applicability of DNA-based methods for olive oil authentication.

Nine olive-specific amplification systems (three previously published by other authors and six lab-designed) were tested by SYBR Green-based quantitative PCR for specific amplification of DNA isolated from leaves of oily species and olive detection in sesame admixtures as relative quantification. For all systems, the limit of quantification and the specificity towards olive oil was determined.

Five amplification systems allowed specific detection of olive DNA: two previously reported (G219/172H and PIP5) and three newly designed (1post-trnE, pre-trnQ and petN-psbM). The lowest LOQ (2.5 pg of olive DNA) was achieved by the 1post-trnE primers. This system was the most sensitive in terms of relative quantification in sesame admixtures, allowing detection of 0.1% of olive DNA. Furthermore, the 1post-trnE system amplified specifically DNA from olive oil. Specific relative quantification, with lower efficiency, was also obtained with the pre-trnQ, G219/172H and PIP5 systems. The results of this work could be used routinely in order to confirm the authenticity of olive oil.

Keywords: Biotechnology, Food Science, Nucleic Acids, Quantitative
Bioanalytical Neurochemistry, Capillary Electrophoresis, Electrophoresis, and Microfluidics

PDMS-Interconnected Microfluidic Systems for Rapid Separations

The complexity of microfluidic systems (MFS) is increasing rapidly as various function parts could be connected in a system. Interconnections play a critical role in the performance of a microfluidic system. Commercial available tube-to-tube connectors are relatively expensive and difficult to further miniaturize. The conventional method of micro-connector fabrication requires precise micromachining and delicate alignment. Thus, the goal is to simplify the fabrication process and interconnection of microfluidic systems. Compared to hard materials, soft materials such as silicone elastomers have become of great interest for microfluidic applications. Reported in this poster is a rapid prototyping method to fabricate poly(dimethylsiloxane) (PDMS) interconnectors in a capillary electrophoresis (CE) system. Owing to PDMS inherent properties: elasticity, optical transparency, and suitability for prototyping, the PDMS fabricated devices have the advantages of ease to fabricate, ease to use, minimal dead volume and excellent sealing. Our studies have shown that these miniaturized interconnectors accommodated tubing connection and provided visible trouble shooting. In particular, the needle-to-capillary connection tolerated up to 120 psi in back pressure. According to dynamic tests, a response time of 18 seconds was achieved in a two-branch CE system. Overall, the rapidly prototyped PDMS interconnectors are reusable, inexpensive, convenient for connection, and reliable when integrated with the CE detection system. Therefore, these robust interconnectors are suitable for rapid separations in microfluidic systems and could be further miniaturized in an integrated device.

Keywords: Analysis, Capillary Electrophoresis
Application Code: Neurochemistry
Methodology Code: Capillary Electrophoresis
The development of cost effective methodologies for precise single molecule protein localization to specific sites is important for the development of various devices with a wide range of biomedical and nano-technological applications. Specifically, precise orientation of proteins on a surface will allow for the production of more sensitive and specific on-chip, microfluidic biosensing devices. We present a bench top technique that utilizes colloidal lithography in order to precisely pattern proteins with nano resolution on glass microscope slides. Patterning as small as 15 nm were achieved. Atomic force microscopy was used to confirm the high resolution and uniformity achieved by this fabrication technique.
Mass spectrometry imaging (MSI) generates multidimensional datasets that combine chemical identity and spatial information. It detects and localizes multiple molecules in a single experiment without prior knowledge, making it useful for de novo analysis of complex biological systems. In this work, matrix assisted laser desorption ionization (MALDI) is used to image peptides in the nervous system of the planarian Schmidtea mediterranea. Because of stem cells distributed throughout their body, these flatworms have the ability to regrow into complete animals from even small severed sections, making them useful models for studying regeneration. We have previously identified the prohormone complement of this model using genomics, transcriptomics, and peptidomics approaches [Collins et al., PloS Biol. 2010, 8, e1000509]. We are now examining if these peptides are involved in the regeneration process using MALDI-MSI. To image, planarians were first chilled, embedded, and cryosectioned. Interfering chemical species were then removed with a series of rinses. Care was taken to reduce the number of rinse-dry cycles, which can cause tissue sections to disintegrate. Mass spectrometry images show several ions localized on the pharynx. Distributions are also observed along the side, consistent with the location of the planarian nervous system. Chemical identifications were done through mass matching with HPLC MS/MS results obtained from previous work [Collins et al., PloS Biol. 2010, 8, e1000509].

**Keywords:** Imaging, Mass Spectrometry, Neurochemistry, Sample Preparation

**Application Code:** Neurochemistry

**Methodology Code:** Mass Spectrometry
The pervasiveness of neuropeptides as signaling molecules in the brain and nervous system, along with their implication in multiple disease states, underscores the importance of understanding neuropeptide release profiles of healthy and diseased neurons. Morphological and electrophysiological studies have shown that neurons respond to specific mechanical environments. However, studies linking specific neuropeptide release studies based on changes in the mechanical environment are lacking. To optimize neuronal growth in microfluidic devices, we have cultured DRG neurons isolated from Sprague-Dawley rats in polydimethylsiloxane (PDMS) devices containing 1 to 3 mm diameter wells. The initial analysis shows that low density culture of DRG neurons in restricted space provides enough material for neuropeptide release to be analyzed using mass spectrometry. To study the chemical peptide profile of the low-density neuronal cultures at different stages of their development and under different amounts of applied, we coupled these microfluidic devices with off-line matrix-assisted laser desorption/ionization mass spectrometric (MALDI-MS) analysis. By using the microfluidic/mass spectrometry neuropeptide measurement protocol, along with a mechanical strain system, we are able to measure neuropeptide release from low-density neuron cultures as a result of changes in their mechanical environment. The high complexity of the observed peptide profiles with a large number of signals detected in peptide molecular mass region indicates the success of our platform, but also underscores the need for further studies. Funding: NIDA DA018310, NINDS NS031609, NSF DMI 0328162, and NSF 0965918.

**Keywords:** Lab-on-a-Chip/Microfluidics, Mass Spectrometry, Neurochemistry, Peptides

**Application Code:** Neurochemistry

**Methodology Code:** Microfluidics/Lab-on-a-Chip
The recent emergence of graphene quantum dots (GQDs) promises to circumvent the limitations (toxicity and lack of water solubility) of their inorganic counterparts due to their strong photoluminescent activity and water solubility. These physical properties of GQDs, along with an emission maximum at 460 nm that is largely independent of excitation wavelength (300-420 nm), warrant a thorough exploration of their potential utility as analytical fluorescent probes. The objectives of this work are thus two-fold: (i) to develop efficient and versatile synthetic preparations of GQDs, and (ii) to employ these materials in sensitive assays for “hard-to-access” analytes, which might otherwise lack spectroscopically active functionalities for detection. This work will employ capillary electrophoresis (CE)-based methods for the characterization of GQDs and for the determination of GQD-modified analytes. Preliminary experiments employed a ‘bottom-up’ method for GQD synthesis, involving the carbonization of an organic precursor (e.g. citric acid) followed by neutralization with NaOH solution. Although CE studies by others revealed the influence of buffer pH on the mobility of graphene oxide nanoparticles, the appearance of numerous unresolved zones detected by UV absorbance provided insufficient characterization of the particles. Thus, in the present work, we will employ the polymer modified capillary transient isotachophoresis “PectI” method originated by our lab in order to characterize our GQDs based on their surface functionalities and size. Further, the initial application of our GQDs as probes for the primary explosive triacetone triperoxide (TATP) showed a proportional increase in GQD emission relative to added analyte concentration, so employing GQDs as analyte probes in PectI or other CE-based separation methods will facilitate rapid, inexpensive, and highly sensitive assays for important and varied classes of analytes.

Keywords: Capillary Electrophoresis, Characterization, Method Development, UV-VIS Absorbance/Luminescence
Application Code: Nanotechnology
Methodology Code: Capillary Electrophoresis
### Session Title
Bioanalytical Neurochemistry, Capillary Electrophoresis, Electrophoresis, and Microfluidics

### Abstract Title
Targeting Membrane Bound Proteins with Methylated Aptamers

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### Co-Author(s)
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### Abstract Text
Aptamers are oligomers that offer great specificity for target binding while exhibiting minimal immunogenicity. Traditionally aptamers are the standard five bases, but with increased interest in aptamers unusual or modified bases are beginning to be investigated, affording a larger selection of aptamers. It is possible to modify specific DNA bases by adding methyl groups by using methyltransferase. The increased hydrophobicity could increase affinity for several targets, including membrane proteins. Standard pools of aptamers and methylated pools of aptamers are going to be used to bind to a membrane protein. During electrophoresis free and bound aptamer separate into distinctive peaks. Coupling fluorescently labeled aptamers with a capillary electrophoresis-laser induced fluorescence system the methylated aptamers can be evaluated against standard unmethylated aptamers.

### Keywords:
Bioanalytical, Capillary Electrophoresis, Nucleic Acids

### Application Code:
Bioanalytical

### Methodology Code:
Capillary Electrophoresis
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 over a period of several days in a microfluidic device consisting of parallel channels fabricated in a poly(dimethylsiloxane) microchip sealed over a polystyrene base. 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 be used to investigate the metabolic pathways that become damaged by hyperglycemia which will aid in the elucidation of potential therapeutic targets.
Bioanalytical Neurochemistry, Capillary Electrophoresis, Electrophoresis, and Microfluidics

Integrating Microscale Enzymatic Reactions with Capillary Electrophoresis

Amyloglycosidase catalyzes the hydrolysis of starch to release smaller sugars and is the major catalytic machinery for the breaking glycosidic bonds. Amylases comprise 18% of the total industrial protease enzyme market[1] for applications that include textiles, detergents, starch, baking, ethanol production and animal feed. In addition, physiological levels of amylase are of clinical importance to diabetes, pancreatitis, and cancer research. Amyloglycosidase performance is described by the catalytic efficiency as well as the catalytic activity. 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. Previously, capillary electrophoresis coupled to mass spectrometry has been utilized to evaluate the performance of amyloglucosidase.[2] The prior work is expanded to utilize capillary electrophoresis separations with alternative detection modes, such as laser induced fluorescence and colorimetric detection. Capillary electrophoresis is used to assess amyloglycosidase and to study the effect of reaction conditions on the Michaelis-Menten Constant. Experimental values are compared with literature values. System specific optimization, method development and validation are reported. The method is transferred to a portable microfluidic device.

References:
Abstract Text
Infrared (IR) spectroscopy directly probes the structural degrees of freedom of molecules and can resolve states that fluctuate on fast timescales. However, the spectral congestion inherent to protein spectra limits its application to spatially resolved investigation of protein function. To alleviate this problem, we incorporated into proteins vibrational probes with spectrally isolated frequencies and local-mode character that make possible rigorous analysis of protein environments and dynamics with IR spectroscopy. In particular, heme-bound carbon monoxide and selectively incorporated cyano-labeled amino acids were introduced as probes of cytochrome P450. IR spectroscopy was then utilized to investigate the local electrostatics and conformational heterogeneity at sites in the protein active site and substrate access pathway. In further studies, the cyano-labeled cytochrome P450 will be characterized with two-dimensional IR spectroscopy to examine the contribution of protein flexibility to the specificity of its catalytic activity.

Keywords: FTIR, Protein, Spectroscopy, Vibrational Spectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Bioanalytical: Vibrational Spectroscopy

A Novel, Fluorescence-Based Assay for Determining MicroRNA Concentration in Solution

MicroRNA’s (miRNA’s) are difficult to quantify accurately because of their small size and often low concentration in RNA samples. Spectrophotometer A260 readings require large quantities of sample, typically 500 [micro]L or more, lack sensitivity and cannot differentiate between intact or degraded RNA. While the NanoDrop Spectrophotometer requires only 1-2 [micro]L of sample, the lower detection limit of the instrument is 2 [micro]g/mL. Fluorescence-based RNA quantification assays are more sensitive, but often detect miRNA poorly. The new Qubit[registered] and Quant-iT[circumflex O] microRNA assays detect small RNA (17-25 nt or bp) with a lower detection limit of 50 ng/mL in the sample and require only 1-20 [micro]L. Both use a fluorogenic dye that exhibits >200-fold enhancement when bound to small RNA. The Qubit[registered] assays are designed for low-throughput (<20 samples) and detection on the Qubit[registered] 2.0 Fluorometer; the Quant-iT[circumflex O] assays are designed for high-throughput (>20 samples) and detection on a fluorescence microplate reader. The assays accurately detect miRNA and small interfering RNA (siRNA) but do not detect free nucleotides, and therefore can distinguish small RNA from completely degraded RNA. Additionally, only about 20 - 30% of large RNA (>1000 nt’s), including ribosomal and messenger RNA, are detected by these assays. Although the reagent is not exclusively selective for miRNA, we have been able to reproducibly measure as little as 0.5 ng of miRNA in pure samples. The Qubit[registered] and Quant-iT[circumflex O] microRNA assays have a dynamic range of 5-500 ng/mL miRNA in the assay tube and accurate results can be obtained for initial sample concentrations ranging from 50 ng/mL to 100 [micro]g/mL. The improvement in accuracy gained by using these assays to determine the small RNA concentration in precious RNA samples can lead to greater success rates in subsequent applications such as sequencing and qRT-PCR.

Keywords: Bioanalytical, Biopharmaceutical, Fluorescence, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Fluorescence/Luminescence
Biosensing utilizing Localized Surface Plasmon Resonance (LSPR) offers label-free, sensitive, and facile detection (1). Recently, a large number of complex nanoparticles have been synthesized in solution, such as nanostars (2), nanocresents (3), nanorods (4), and nanocubes (5). Although many of these plasmonic nanoparticles are expected to be very useful towards biosensing applications, most have not yet been applied towards these goals. This is mainly due to the presence of capping agents on the surface of the nanoparticles, preventing the binding of biological molecules of interest. In order to allow plasmonic nanoparticles of complex shapes to be used in biosensing applications, we have developed conditions to remove the capping agent, polyvinylpyrrolidone (PVP) from gold nanostars (Figure 1A), and followed this removal using UV-Vis and surface enhanced Raman spectroscopy (SERS). The three plasmon absorptions of the gold nanostars all showed substantial blue shifts (~80 nm) upon PVP removal (Figure 1B). In addition, the SERS enhancement for benzenethiol coated nanostars without PVP show a dramatic improvement over nanostars with PVP (Figure 1C). This capping agent removal methodology shows great promise towards utilizing other plasmonic nanoparticles of complex shapes in biosensing applications.


Keywords: Biosensors, Surface Enhanced Raman, UV-VIS Absorbance/Luminescence, Wet Chemical Methods
Application Code: Bioanalytical
Methodology Code: UV/VIS
Bioanalytical: Vibrational Spectroscopy

Development of a SERS Technique for the Quantitative Analysis of Bidentate Compounds

Surface Enhanced Raman Spectroscopy (SERS) is a technique that has been commonly used to identify compounds in extremely small concentrations. However, the use of this technique as a method of quantitative analysis has not been fully explored. By adding an internal standard to a SERS system, this project aimed to make quantitative analysis both possible and simple. The initial species examined was dopamine, a neurotransmitter being investigated as a treatment option for Parkinson’s disease. In the blood stream during a treatment cycle, dopamine is present in very small concentrations, and needs to be measured with great precision and accuracy. Measuring dopamine in this way is one of many applications that this project’s set up is designed be able to address. The intensities of the SERS peaks associated with the dopamine and the intensities of the peaks associated with the internal standard on the docking molecule was used to generate a calibration curve over a range of concentrations of dopamine. In comparing the ratio of the intensities to the concentrations of dopamine at which they were taken, a calibration curve was obtained. With high R2 values, indicating good fits for the curves, and short error bars, indicating a sufficient level of precision, the data obtained asserts that our quantitative application of SERS is a possibility. With the successful calibration curve, we are given the opportunity to pursue variants of the system developed in this project. Complexing the docking molecule with nickel instead of iron would allow us to look at histidine compounds and a zirconium complex would allow us to look at phosphate groups. This project has these and many other options for future work.

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Abstract Text

Keywords: Absorption, Detection, Raman, Spectroscopy

Application Code: Bioanalytical

Methodology Code: Vibrational Spectroscopy
Nanolipoprotein particles (NLPs), also known as reconstituted high density lipoproteins (rHDLs), are discoidal self-assembled particles. They are commonly used as model membranes for the purpose of studying membrane proteins and their interactions with other biomolecules. In addition, the use of NLPs as an in vivo delivery vehicle has recently been explored for a variety of applications, including vaccine applications. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was used to investigate the secondary structure of the apolipoprotein apoE422K; which was used in the construction of these NLPs. Changes in the secondary structure as a result of temperature changes will be reported. Due to the amide I band consisting of smaller overlapping bands produced by the secondary structure elements, deconvolution of the amide I allows for monitoring changes in the apoE422K's secondary structure.

**Abstract Text**

**Keywords:** FTIR, Immobilization, Vibrational Spectroscopy

**Application Code:** Materials Science

**Methodology Code:** Vibrational Spectroscopy
Inflammation and the attendant inflammatory responses to exogenous stressors are among the important mechanisms in human adverse outcome pathways (AOPs) structures. Scientific evidence suggests that inflammation is associated with human health effects and health endpoints, yet most studies have focused on human populations that are already considered “unhealthy”. As such, it is pertinent to measure inflammatory biomarkers in human biological media with an appropriate sensitivity and specificity for an environmental or low-level exposure. To this end, we used Meso Scale Discovery’s (MSD) MUTIL-SPOT Human TH1/TH2 Cytokine10-Plex Assay Ultra-Sensitive Kit (Gaithersburg, MD) to evaluate the use of this methodology for the investigation of interleukins (1, 2, 4, 5, 8, 10, 12p70 and 13), IFN- and tumor necrosis factor- in human plasma, exhaled breath condensate and urine. We have found that the MSD instrumentation and the TH1/TH2 cytokine 10-plex method have sufficient sensitivity and specificity for assessing the target biomarkers in a nominally healthy population. The ten cytokines in the panel had limits of detection ranging from 0.054-2.3 ng/mL, method blank coefficient of variations (CVs) that remained below 10% for >98% of the results, and average between and within plate CVs of 17.3 and 14.6, respectively. Our results demonstrate that the methodology performs suitably to identify low-level inflammatory responses in human blood, exhaled breath condensate and urine in “normal” healthy subjects.
Modified starch production via esterification provides a commodity in large quantities destined for many everyday applications. The degree of substitution is typically determined by titration or NMR of the bulk product. For the purpose of assessing the uniformity on a granule to granule basis, we previously performed single granule census via FT-IR microspectroscopy ratioing the ester carbonyl to carbohydrate absorption band areas. The octenyl succinate product approved for human consumption as an emulsifying agent at a 3% level showed that the spectra of several out of 100 granules did not exhibit the ester carbonyl at 1724 cm⁻¹. However, at high substitution (25%), e.g. spectra of 99% of the individual granules were classified as having been modified. This presentation concerns the acetate ester of starch used in large quantities in pharmaceutical products for time release and for numerous industrial purposes. Transmission infrared microspectroscopy of flattened starch granules is demonstrated as a quantitative method for determining the degree of substitution specifically for highly modified starches that are less suited for analysis with NMR methods due to limited solubility.

Keywords: Carbohydrates, Microspectroscopy, Process Control
Application Code: Quality/QA/QC
Methodology Code: Vibrational Spectroscopy
Surface-enhanced Raman scattering (SERS) is a powerful and non-destructive technique providing fingerprint information about molecular structures brought into the close vicinity of or in contact with noble metal surfaces. Besides qualitative analysis, it also provides quantitative analysis of the molecule of interest. In this study, semi-quantitative analysis of proteins regardless of their size, charge and molecular structure was performed. Using various blood proteins including HSA, transferrin, IgG, and IgA, the use of SERS for quantitative determination of proteins by a fast, simple and label-free method was demonstrated. Synthesis of silver nanoparticles (AgNP) using hydroxylamine is very fast. Nanoparticles are synthesized immediately upon hydroxylamine addition to silver nitrate solution. In this study, proteins were mixed with silver nitrate solution of which concentration was much higher than the amount required for AgNP colloid synthesis. Then, hydroxylamine was added. Due to excess silver nitrate, bands in the acquired SERS spectra were of the byproducts of the synthesis and nitrate ions rather than proteins, and intensity of the bands decreased as the protein concentration increased. A detection limit down to 10 nM and a quantification range between 100 nM and 10 nM were easily achieved using this simple, fast and label-free approach. The authors acknowledge the financial support from Yeditepe University and The Scientific and Technological Research Council of Turkey (TUBITAK) during the course of this project.
Lipid identification is important for a variety of systems, such as lipid vesicles used for drug delivery and microvesicles associated with disease. We have developed a flow cell detector that incorporates a planar surface enhanced Raman substrate to enable ultrasensitive detection in flow. The flow cell builds upon flow cytometry and hydrodynamic focusing to confine the molecules and particles exiting a capillary within the flow chamber near the SERS active surface, allowing for more interactions with the nanostructures and consequently more enhancements. We have successfully coupled this SERS flow detector with capillary electrophoresis, enabling online CE-SERS detection. We present results showing lipid vesicles that migrate electrophoretically through the capillary and are detected by SERS. Using this setup, we are able to separate and detect lipids expressing different polar head groups and different surface charges. The SERS signal generated from the different vesicles yields a different spectrum, allowing for easy and rapid identification of the lipid vesicle species.

Keywords: Capillary Electrophoresis, Lipids, Surface Enhanced Raman, Vibrational Spectroscopy
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Abstract Text

In this work, silver nanoparticles (AgNPs) decorated ZnO/Fe3O4 composite structure is prepared as surface-enhanced Raman scattering (SERS) substrate for the analysis of uric acid in urine sample. To prepare this substrate, magnetic nanoparticles were first synthesized by coprecipitation of Fe (II) and Fe (III) solutions with ammonium hydroxide. Precipitation of Zn (NO3)2 with NaOH in the presence of magnetic nanoparticles resulted in a composite structure with magnetic and catalytic properties acquired from the counterpart components, Fe3O4 and ZnO respectively. Photochemical reduction method has been employed to deposit AgNPs by exposing a reaction mixture containing AgNO3 solution (dissolved in ethylene glycol) and ZnO/Fe3O4 composite to UV light. Optimum reaction conditions were set out by variation of concentration of AgNO3 and UV irradiation time. Formations of these structures are confirmed from XRD and EDX data. To examine the performance of the prepared substrate for SERS activity, the particles were dispersed in methanoic solution of para nitrothiophenol (pNTP) and aqueous solution of uric acid and recovered by cylindrical magnet for Raman scanning. Highly reproducible and good Raman activity was observed and the substrate has been further used for determination of uric acid in urine sample.

Keywords: Detection, Raman, Surface Enhanced Raman, X-ray Diffraction
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Approximately 17 million units of blood are collected each year; statistics show this number to be continuously increasing. To be used in a clinical setting, blood needs to be typed. This process generally involves the mixing of human erythrocytes with separate antibodies and looking for agglutination in the presence of these reagents. The objectives of this study were threefold: 1) to acquire FT-IR spectra from human blood cells, 2) to analyze by classic spectroscopic methods the FT-IR spectral features of blood samples typing as Group A, Group B, Group AB and Group O, and (3) to employ multivariate techniques including back-propagation neural network and vector machine algorithm (SVM) for classifying the spectra of blood samples by their groupings. A total of 245 samples were analyzed. Of these samples, 61 were typed as group A, 61 samples as group B, 62 typed as AB, and 61 belonged to group O. The evaluation of spectra by multivariate analysis revealed that the region between 900 cm$^{-1}$ to 1600 cm$^{-1}$ offered the most information, with an overall accuracy rate of 82%. The highest accuracy was achieved with samples that typed group B, correctly identified in 95% of samples by SVM. This was followed by group AB, with 83% correctly identified. For samples that typed as blood groups O and A, SVM analysis yielded accuracy rates of 79% and 70.5%, respectively. These results warrant further investigation, as FT-IR microspectroscopy may prove to be a valuable tool for analyzing blood glycoproteins.
Understanding protein dynamics is an integral part in elucidating the correlation between protein structure and function. Plastocyanin and cytochrome f are two proteins involved in the photosynthetic electron transfer chain. In order for electron transfer to occur, the two proteins form a loosely bound encounter complex which can then evolve into a well-defined complex allowing for electron transfer. This serves as a perfect model complex for studying dynamic structures and their adaptation for specific biological function. Specific regions of the two proteins can be explored using C-D labeled amino acids to probe changes in the local environment during the course of their transient interaction. These non-perturbative probes exhibit frequencies in the transparent frequency window for proteins (1900 – 2500 cm⁻¹) allowing study via linear FTIR spectroscopy of the residue of interest, void of any congestion from other protein vibrations.

Cyanobacteria, Nostoc sp. PCC 7119, will serve as the complex of interest. Proposed future work will be to incorporate C-D bonds into amino acids located in the electrostatic and hydrophobic patch between Pc and cyt f as well as their respective redox centers. Results from these experiments can then be extended to cyano (CN) labeled amino acids in the same locations for use in 2D-IR vibrational echo experiments.

Abstract Text

Co-Author(s)
Metabolomics is one of the important fields of research in the ‘omics’ concerned with the study of low molecular weight compounds (metabolites) within a biological system and investigating their differences between natural and perturbed samples such as cells, organs and tissues. Rapid identification and discrimination of biological samples based on metabolic differences and physiological status is highly desirable. Surface-enhanced Raman spectroscopy (SERS) is a promising technology for metabolomics analysis due to its high-sensitivity, label-free and fingerprint analysis. We have developed a novel SERS substrate named “gold nanocoral (GNC)”, having gold nanospheres on the crest of boehmite. The GNC provides high-uniformity over a large area as well as ease of production. Since GNC is formed on a transparent substrate, light irradiation and Raman scattering measurements can be carried out by approaching through the substrate. It makes possible a SERS sensing and imaging without any obstruction by the sample. To evaluate the ability of metabolomics analysis with GNC, we have conducted the SERS imaging of adenylates in a mouse brain with ischemia. The result shows the apparent distribution of adenylates compared with a normal brain sample and suggests the method has a great potential for metabolomics analysis tools.

Keywords: Biosensors, Biospectroscopy, Imaging, Surface Enhanced Raman
Application Code: Bioanalytical
Methodology Code: Biospectroscopy
In last decades Raman spectroscopy has attracted considerable interest as a potentially clinical tool for nondestructive and real-time diagnosis of diseases, including breast cancer, dominantly due to its highly specific information regarding molecule features. In this study, frozen sections (about 6 µm thickness) of breast tissues collected from 56 patients were provided by the Department of Breast Surgery, the First Hospital of Jilin University. The tumor locating at HE sections were detected at the same spots of its frozen contiguous sections by Raman spectroscopy (shown in scheme 1).

The mean of 619 Raman spectra from frozen sections of breast tissues including normal breast tissues (NB), fibroadenoma (FD), atypical ductal hyperplasia (ADH), ductal carcinoma in situ (DCIS), and invasive ductal carcinoma (IDC). We can clearly find that the spectral features of tissues, peaks at around 1120, 1262, 1300, 1442 and 1745 cm⁻¹, are attributed to vibrational modes of lipid. Additionally, the prominent bands at 1004, 1033, 1610 and 1658 cm⁻¹ are related to vibrational modes of protein. Moreover, the peaks at around 1157 and 1525 cm⁻¹ represent DNA and carotene, respectively.

Significant differences in Raman spectra of various breast tissues can be summarized as following: characteristic of the C-S stretching mode of cystine, located at 665 cm⁻¹, only presents in FD. Regarding to ADH, the unique feature of 1612 cm⁻¹, is related to Amide I of protein. Carotene band at 1525 cm⁻¹ has strong presence in DCIS and IDC but almost can not be observed in other tissues, which confirms that Raman spectroscopy has potential to further realize the early diagnosis of breast cancer in situ.

Fundings: NSFC Grant Nos. 81202078, 21073073 and 91027010, and National Instrumentation Program (NIP) of the Ministry of Science and Technology of China No. 2011YQ03012408.

Keywords: Bioanalytical, Biomedical, Raman
Application Code: Biomedical
Methodology Code: Vibrational Spectroscopy
Radiopharmaceuticals are radioactive substances used in positron emission tomography (PET) to diagnose, treat, or prevent diseases. They contain a radioactive isotope, a so-called radionuclide, attached to a biologically inert or active molecule. The radionuclide is unstable and therefore radioactively decays under emission of a positron. This positron encounters an electron and annihilates it. As a consequence, two photons that can be sensitively detected are emitted in opposite directions.

After the radiopharmaceutical is concentrated in the tissue of interest, the patient is placed in the PET scanner. By tracking the photons, computers with sophisticated software generate three-dimensional images of the photon source. This allows to study physiological, biochemical, and pharmacological functions at a molecular level. Illnesses such as cancer, cardiovascular disease, and even neurological disorders can be detected long before symptoms appear. The most commonly used short-lived, cyclotron-produced radionuclides in radiopharmacy are 11C, 13N, 15O, and 18F. As the fluorine atom is similar in size to the hydrogen atom, it acts as a pseudohydrogen and is hence ideally suited for replacing hydrogen atoms in organic molecules such as dopamine, glucose, or choline.

Quality control of the short-lived radiopharmaceuticals is challenging, because of the tough time limits, the radiation issue, and the near nanomole radiotracer quantities. This poster looks at a rugged and versatile multichannel radio ion chromatography system that controls the production of the radionuclide [18F]fluoride (precursor) and the two radiotracers synthesized from it, [18F]fluorodeoxyglucose and [18F]fluorocholine, in accordance to pharmacopoeial regulations.
Abstract Text

Purification of proteins can often show varying recovery values as a direct result of the presence of carryover within an automated HPLC purification system. During method development, time should be spent to evaluate the full system, tubing, and accessories in a worse-case scenario to identify and remove the carryover source. Bovine Serum Albumin (BSA) is a standard protein that has been frequently labeled a problem protein for many laboratories performing ion exchange protein purification. As a result, using BSA can be advantageous as a worse case sample for carryover testing following purification. When carryover is identified, the investigation process is performed systematically to remove the root cause of the carryover. Carryover can be solved by a combination of modifications to system and accessory tubing materials, chemistry or solvents, flexible software programming, and physical changes to system plumbing. The workflow and supporting data presented in this application illustrates how a protein sample comprised of BSA and Ovalbumin can be used successfully to develop a robust and optimized method for an automated purification system that removes carryover and generates consistent recovery values prior to routine protein purification.
The ability to monitor and control variability of inorganics in raw materials used for cell culture media is important for consistent cell growth and therapeutic protein quality in bio-pharmaceutical production. Routine investigations of raw materials have been hindered due to traditional metal analysis techniques such as inductively coupled plasma – mass spectrometry and atomic absorption spectroscopy, which are both time and labor intensive. Here, the use of a portable x-ray fluorescence (XRF) spectrometer to quickly (no sample preparation) and easily (click of a button) perform metal analysis of common ingredients used in media preparation including hydrolysate powders, chemically defined powders, and liquid supplements will be presented. To illustrate the utility of the XRF spectrometer, cases studies demonstrating sub-ppm variability of Zn and Cu, as measured with the XRF, in lots of hydrolysate with cell culture performance are discussed. In addition, a proactive strategy incorporating XRF analysis for high throughput screening of raw materials will be given.

Keywords: Biopharmaceutical, Elemental Analysis, X-ray Fluorescence
Application Code: Biomedical
Methodology Code: X-ray Techniques
Alpha1-acid glycoprotein (AGP) is one of the most important acute phase proteins and is the principal binding protein for basic and neutral drugs in serum. AGP was used in this study as a ligand to prepare affinity microcolumns for use in ultrafast affinity extraction. These columns were then used to examine the binding and dissociation kinetics of various drugs with soluble AGP. When a drug-protein mixture was injected onto the AGP microcolumn, the free fraction of the drug was extracted and retained by the column for analysis and measurement. The column size and flow rate were optimized in these experiments to achieve a sample residence time of less than a few hundred milliseconds to avoid the dissociation of the drug from proteins in the sample during the measurement of free drug fractions. Propranolol, Imipramine, lidocaine were used as examples of drug targets in this study. The free drug fractions and binding constants that were determined by this approach were comparable to the results of reference methods. The corresponding equilibrium constants were also in close agreement with the literature values. It was determined from these results that the AGP microcolumns could be useful tools for studying the drug-protein interactions or for screening new drug candidates.
Direct analysis in real time (DART) with high-resolution mass spectrometry has excellent potential for identifying small molecules on and in cultural heritage objects and historic materials. While DART can be used without sample preparation, hydrolysis of proteins and in situ derivatization yields additional information about the composition of such materials. Proteinaceous glues on historic ceramics, and similar binding media in paints can be differentiated based on their amino acid content, which can be determined rapidly and simply with DART-MS. Organic colorants from natural dyes can be directly identified in wool and cotton textile fibers simply held in the ion source. Adjusting various parameters – MS orifice voltage and DART gas temperature – makes it possible to identify blood residues and to differentiate isomeric organic dye colorants. We report here on progress in utilizing DART-MS to characterize several different types of historic materials.
Textiles are rare in the archaeological record. When they are found, they are often poorly preserved and contaminated from the burial environment. Natural red dyes in such textiles were derived from plants and even insects, and identifying the sources of dyes tells archaeologists about human behavior in the past. DART-MS can be used to identify dye colorant compounds in cotton and wool textile fibers. The samples available from archaeological textiles for analysis and dating are often only tiny fragments, coated with soil and even plasticizer from storage bags. In order to identify dyes in such samples by DART-MS, cleaning is necessary to remove these contaminants. We are investigating the effects of cleaning protocols developed for radiocarbon dating on reference samples of wool dyed with madder (Rubia tinctoria), bedstraw (Galium verum) and bloodroot (Sanguinaria canadensis), to develop a reliable method for identifying the dye colorants from these plants by DART-MS. The results of this study will be applied to fragments from archaeological textiles.

**Abstract Text**

Textiles are rare in the archaeological record. When they are found, they are often poorly preserved and contaminated from the burial environment. Natural red dyes in such textiles were derived from plants and even insects, and identifying the sources of dyes tells archaeologists about human behavior in the past. DART-MS can be used to identify dye colorant compounds in cotton and wool textile fibers. The samples available from archaeological textiles for analysis and dating are often only tiny fragments, coated with soil and even plasticizer from storage bags. In order to identify dyes in such samples by DART-MS, cleaning is necessary to remove these contaminants. We are investigating the effects of cleaning protocols developed for radiocarbon dating on reference samples of wool dyed with madder (Rubia tinctoria), bedstraw (Galium verum) and bloodroot (Sanguinaria canadensis), to develop a reliable method for identifying the dye colorants from these plants by DART-MS. The results of this study will be applied to fragments from archaeological textiles.
Direct analysis in real time (DART) mass spectrometry has potential for rapid characterization of residues in and on archaeological materials, often without the need for extraction and sample preparation. These residues may provide insight into people’s behavior in the ancient past, including what they ate and drank and what plants they used. Residues change over time through oxidation, loss of water-soluble components during washing, and contamination from burial and handling. We are investigating how nicotine residues in historic smoking pipes from St. Mary’s City, Maryland have changed through time. To understand how residues change as they age and decompose, we are studying tobacco pipes that have been excavated and not cleaned, as well as pipes that have been cleaned and processed by the museum. Nicotine and several of its oxidation products are readily observed in replica residues analyzed with DART-MS, even after the ceramic surfaces have been cleaned. We report here our progress with historic materials from the 17th-century.
Mass spectrometry imaging (MSI) is a powerful technique in providing chemical and spatial information of molecules of interest simultaneously. We report a systematic performance comparison of two porous silicon-based (pSi) MSI methods; matrix enhanced surface assisted laser desorption/ionization (ME-SALDI) and nanostructured initiator mass spectrometry (NIMS). ME-SALDI, an extension of desorption/ionization on silicon (DIOS), utilizes a thin layer of matrix to aid in the desorption/ionization of small molecules of interest. NIMS, on the other hand, uses a thin polymer coating, “an initiator”, to assist with analyte desorption/ionization. In this study, 2,5-dihydroxybenzoic acid (DHB) and 1,3-bis(heptadecafluoro-1,1,2,2-tetrahydrodecyl)tetramethyldisiloxane (BisF17) were used as the desorption/ionization aid for ME-SALDI and NIMS, respectively. Several biologically relevant small molecules were used to examine the stability and reproducibility of these two ionization methods, which showcased the versatility of both techniques. Different substrate preparation conditions were compared between n- and p-type pSi substrates. The current density used during etching and initiator coating time was varied. The signal-to-noise (S/N) and the mass resolutions of MS peaks of interest were calculated and used to assess the desorption/ionization efficiency of two pSi-based LDI methods. Additional studies were conducted to compare MS behaviors under positive or negative modes.

Preliminary findings showed that ME-SALDI initially has better detection sensitivity when the substrate is freshly prepared; however a steady decrease in S/N is observed over a two week time period. In addition, small matrix peaks are still observed in ME-SALDI, albeit at much reduced intensities than those in traditional MALDI. In the same two-week time period, n-type NIMS exhibited much better stability, but slightly lower signal sensitivity than ME-SALDI. Additionally, n-type NIMS also showed better spot-to-spot and batch-to-batch reproducibility compared to ME-SALDI. This increased stability and reproducibility of n-type NIMS substrate can be attributed to the inert polymer coating that protects the porous silicon surface from oxidation. Similar behaviors have been observed for p-type NIMS as well. Upon the systematic comparison of these two pSi-base ionization methods with internal standards, future work will aim to use MSI to compare mouse brain tissues between ME-SALDI and NIMS.

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

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
Combining Fibrinogen-Conjugated Gold Nanoparticles with a Cellulose Membrane for the Mass Spectrometry-Based Detection of Fibrinolytic-Related Proteins

In this paper, we describe a pulsed-laser desorption/ionization mass spectrometry (LDI-MS) approach for the detection of plasmin with subnanomolar sensitivity through the analysis of gold (Au) clusters desorbed from fibrinogen-modified gold nanoparticles (Fib\textsubscript{Au} NPs) on a mixed cellulose ester membrane (MCEM). The mechanism of action of this probe is based on the plasmin-mediated cleavage of the Fib\textsubscript{Au} NPs and the reduced interaction between Fib\textsubscript{Au} NPs and MCEM. The Fib\textsubscript{Au} NPs were deposited onto the MCEM to form a highly efficient background-free surface-assisted LDI substrate. Under pulsed laser irradiation (355 nm), the cleaved Fib\textsubscript{Au} NPs decreased the adsorbed on MCEM. As a result, the intensities of the signals of the Au clusters decreased in the mass spectra. This approach provided a highly amplified target-labeling indicator for the analysis of plasmin. Under optimized conditions, this probe was highly sensitive (limit of detection: ca. 0.1 nM) and selective (by at least 1000-fold over other enzymes and proteins) toward plasmin; it also improved the reproducibility (<5%) of ion production by presenting a more-homogeneous substrate surface relative to surface-assisted laser desorption/ionization mass spectrometry (SALDI-MS) analysis, thereby enabling LDI-MS to be used for the accurate and precise quantification of plasminogen in human serum in the presence of urokinase (an activator that converts plasminogen to plasmin). Relative to conventional assays, this new probe for plasmin offers the advantages of high sensitivity and selectivity and high throughput, with great potential for practical studies of fibrinolytic-related proteins.

Keywords: Analysis, Biosensors, Mass Spectrometry, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Analytical tools based on proteomics and lipidomics are being developed to facilitate investigations with high chemical sensitivity, specificity and spatial resolution to carry out studies at the subcellular level. The massive complexity of the nervous system and also the lack of known pathogenic mechanisms and cellular processes underlying neurodegenerative disorders can be carried out with ‘omics’ approaches. The targets are to better understand neurodegenerative diseases such as Alzheimers, Parkinsons and Multiple Sclerosis (MS), an important group of neurological disorders causing serious, chronic, and often irreversible damage to central nervous system.

Two promising label-free imaging techniques, Matrix Assisted Laser Desorption Ionization (MALDI) using a sublimation matrix deposition method developed in our lab provide lateral resolution of a few micrometers and Secondary Ion Mass Spectrometry with submicrometer lateral resolution, particularly for small biomolecules, have been successfully utilized to image and map the cerebral cortex and the corpus callosum of the mouse brain to detect neuropeptides and lipid distribution, which can be useful for investigating the chemical mechanism and changes in biomolecules during the progression of neurological disorders.

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

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
In this study, we developed a label free, ultrasensitive graphene oxide (GO)-based probe coupled with laser desorption and ionization mass spectrometry (LDI-MS) for the detection of oligonucleotides. Through the simple stacking and electrostatic interactions between rhodamine 6G (R6G) and GO, we prepared a nanocomposite, R6G-modified GO (R6G-GO). We found the intensities of the signal of the R6G increased in the mass spectra in the presence of single-stranded DNA (ssDNA) under pulsed laser irradiation (355 nm) of R6G-GO. In addition, the increased signal of R6G was larger in the presence of shorter length ssDNA. Based on the fact that small size oligonucleotides improve the LDI efficiency of R6G on GO, we designed an enzyme amplified signal transduction probing system for detection of microRNA. After the Exonuclease III (Exo III) specifically digest the probe DNA (pDNA) strand from pDNA/microRNA (miRNA) hybridized complexes, the resulted small size oligonucleotide fragments increase the R6G signal in LDI of R6G-GO. As a result, the intensity of the signal of R6G ions increased upon increasing the concentration of targeted miRNA. Coupling these enzyme reaction and R6G-GO with LDI-MS allowed the detection of microRNA at concentrations down to the picomolar regime. We also demonstrated the analysis of microRNA in tumor cells. Furthermore, we applied this R6G-GO probe for the detection of single-nucleotide polymorphisms (SNPs) of the Arg249Ser unit in the TP53 gene. This simple, rapid, sensitive detection system, based on the coupling of functional GO with LDI-MS, appears to hold great practicality for bioanalyses of oligonucleotides and proteins.
Mass Spectroscopy: Bioanalytical and 'Omics

Development of a Quantitative LC-MS/MS Assay for the Simultaneous Quantitation of Acetylcholine, Histamine, and Their Metabolites in Human Cerebrospinal Fluid (CSF) Using sub 2µm HILIC UPLC

Biochemical biomarkers, derived from biological fluids, are often used in drug discovery and development as a way of identifying disease or effectiveness of drug treatment. Acetylcholine (ACh) and Histamine (HA) are highly polar neurotransmitters that act in the peripheral and central nervous system. Given the physiological importance of ACh, HA, and their metabolites in regards to memory and cognition, the ability to measure changes in their physiological concentrations as a function of disease progression or drug treatment make them highly advantageous as potential biomarkers, and therefore, the simultaneous measurement of these biomarkers is a valuable tool that may lead to insight into mechanisms and/or therapeutic treatment of cognitive impairments.

While there are a number of analytical methodologies for quantifying these analytes (GC/MS, HPLC-EC, and LC/MS) there are few in which ACh, HA, and their metabolites have been quantified simultaneously. Additionally, quantitative analysis of ACh, HA, and their respective metabolites, is a persistent challenge due to the demand for high sensitivity, selectivity and the need for fast sample analysis times.

The method described herein demonstrates the simultaneous quantification of ACh, HA, and their metabolites in human CSF in a 96-well format. This application uses a single step sample preparation using a small sample volume, based on a 5X sample dilution with isotopically labeled internal standards, followed by HILIC UPLC-MS/MS analysis using an ultra high efficiency sub 2µm HILIC column to increase analyte sensitivity achieving analyte resolution from endogenous matrices in an analysis time of 2.5 minutes.

Keywords: Bioanalytical, Chromatography, Drug Discovery, Tandem Mass Spec
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Lipidomics on Intact Breast Cancer Cell Lines Using Desorption Electrospray Ionization Mass Spectrometry

The up-regulation of endogenous fatty acid and complex lipid synthesis is a characteristic feature of breast cancer, but their role in carcinogenesis remains largely unknown. Phospholipids mediate important cellular processes such as membrane organization, intercellular communication, cell proliferation, and apoptosis. So, understanding mechanisms of tumor growth requires elucidating the lipid composition of cancer cells as a function of gene expression. In addition, genetic classification of breast cancer cells is important for diagnostics and for the development of personalized treatment regimens. At present, lipidomics of cancer cells is typically carried out using high performance liquid chromatography-mass spectrometry after application of various solvent extraction methods. These approaches involve relatively time consuming separation steps. Herein, we describe a desorption electrospray ionization mass spectrometry (DESI-MS) cell-based workflow that allows direct characterization and classification of breast cancer cell lines having different genetic mutations based on their lipid compositions. In this approach, cultured cells were deposited on microscope slides to form a small pellet of a few millimeters in diameter, which was then analyzed using DESI-MS coupled to an LTQ-Orbitrap XL mass spectrometer. Using this approach, we characterized the lipid compositions of various breast cancer cell lines and showed that there is a general increase in phosphatidylinositols when compared to an immortalized normal cell line. These results agree with previous studies showing that PI3K/Akt pathways are up-regulated in breast cancer, indicating that cell-based DESI-MS assays are powerful tools for probing intrinsic disease mechanisms in vitro.

Keywords: Bioanalytical, Lipids, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Dodecafluoropentane (DDFP) is a volatile (B.P. 29°C) compound that has shown considerable promise as an oxygen delivery agent in animal models of stroke. There is significant interest in monitoring the concentration of DDFP in the extracellular fluid (ECF) space and microdialysis sampling has been effective in the collection of solutes from ECF. However, microdialysis sampling works best with hydrophilic solutes and DDFP is hydrophobic.

In this study, we investigated the use of microdialysis sampling to collect DDFP in vitro directly from a headspace vial (1 L neat) followed by dialysate analysis using GC/MS. The microdialysis probe (CMA 20 PES 100 kDa MWCO) was inserted into the vial through a septum in the cap and the outlet line was directed to a separate, sealed, headspace vial. Samples were collected at 15 minute intervals and the headspace in the second vial was analyzed by GC/MS (column temperature 35 °C, injector temperature 240 °C, isothermal run for 3 minutes) for the detection of DDFP.

Attempts to detect DDFP using water and air as the perfusion fluids in the microdialysis sampling were unsuccessful. However, when a vegetable oil (Enova™) containing primarily diacylglycerols was used as the perfusion fluid, DDFP could be detected in the outlet fluid directed into the second headspace vial. The microdialysis probe retained its integrity despite the passing of diacylglycerols through it for extended time periods. This preliminary work demonstrates that microdialysis sampling techniques can be modified to collect solutes such as the hydrophobic DDFP that are challenging from an analytical chemistry perspective.

Keywords: Analysis, Gas Chromatography/Mass Spectrometry, Headspace, Sampling
Application Code: Bioanalytical
Methodology Code: Gas Chromatography/Mass Spectrometry
Mycobacterium avium spp. paratuberculosis (MAP) is highly pathogenic for ruminants and is suspected to cause chronic inflammatory gut disease in humans. Because of its slow growth diagnosis is difficult and takes time. This study was intended to determine MAP specific VOC (volatile organic compound) signatures from headspace of feces and breath of goats.

Two groups of goats, one infected with MAP and one control group, were included into the study. At 7 stages of infection, samples were acquired from headspace of feces and alveolar breath. VOCs were pre-concentrated from headspace of feces by means of SPME and from breath by means of NTME. Extracted compounds were analyzed by means of gas chromatography and mass spectrometry (GC-MS).

23 substances in feces and 6 substances in breath showed significant differences between infected animals and controls. These patterns included alcohols (60 ppbV - 20 ppmV) and aromatic compounds (1 - 100 ppbV) in breath. Ketones (5 ppb - 5 ppm), furans (1 - 400 ppbV), sulfides (15 ppbV - 30 ppmV) and alkanes (5 - 85 ppbV) were found in headspace of feces. Concentrations of marker substances changed over time and with the infection status.

Micro-extraction-techniques enable effective field sampling for detection of MAP in vivo. VOC-pattern-based analytical methods may, therefore, help to prevent spreading of mycobacteria in livestock through rapid recognition of infection.
Cissampelos owariensis, commonly known as velvet leaf is a multipurpose medicinal plant. The leaves are used in Alternative medicine to correct characteristic symptoms responsible for infertility in women. The essential oil from the dried leaves was obtained by hydrodistillation using two modes of collection into hexane; hourly collection over four hours and a continuous collection for four hours. The fractionation obtained by the hourly collections was carried out to detect some constituents which would be too small to be identified in the 4-hour sample. The five samples were analyzed on GC-MS fitted with HP 5 MS capillary column using a temperature program; 80 degrees (2min), increased at 4 deg./ min. to 240 deg.(5min.). The major constituents are n-hexadecanoic acid (17.7-30.7 %), 2-pentadecanone, 6,10,14-trimethyl (8.5-30-3 %), 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl)ester (0.7-24.4 %) and phytol (12.4-19.5%). Other constituents include isophytol (1.3-6.0 %), caryophyllene oxide (2.8 %), 1H-cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-,[1ar-(1a.alpha.,4a.alpha.,7.beta., 7a.beta.,7b.alpha.,)]- (0.82 %), 3-buten-2-one,4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-, (E-) and isomer (1.04 %), hexadecanoic acid, methyl ester (0.7-4.3 %), 9- octadecenoic acid methyl ester (1.5-6.1 %), methoxyacetic acid, 2-tetradecyl ester (0.42-0.61 %), 2-piperidinone, N-[4-bromo-n-butyl]- (0.7-0.8 %), 13-docosenamide (4.2 %), 3-eicosene, (E)- (1.4-2.7 %) and 1-octadecanesulphonyl chloride (0.4 %). These constituents suggest that the essential oil should exhibit antibacterial, antifungal, anti-inflammatory and insecticidal properties.
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. A major concern, however, is that the two samples can be chemically quite different as the sample for MALDI analysis requires treatment with a suitable matrix prior to analysis. Sensitivity in MALDI depends on the efficiency of extraction and co-crystallization of different species by and in the matrix and also the gas phase basicity of the analyte molecule relative to the matrix. One approach to address this issue that we report here is the use of nanoparticles. Nanoparticle addition has been shown to enhance signal in SIMS analysis although the exact mode of operation is not fully understood. In some cases cationization can occur, but simply aiding charge compensation might be equally important. Nanoparticles can also be used as a substitute for the organic acid matrix normally used in MALDI. Nanoparticles can efficiently capture the energy of the laser thus giving rise to the phase explosion associated with ion desorption in MALDI; however, with additional advantages that small molecules are not swamped by matrix signals and the modification of the sample is minimal. We will present a comparison of SIMS, MALDI, and nanoparticle assisted LDI using test molecules of biological relevance including lipids as well as cultured cells.
The edible nuts from Tetracarpidium conophorum, commonly called African Walnut, are used for the management of breast cancer in Alternative medicine. The nuts which had been boiled were crushed and the essential oil extracted by hydrodistillation, was collected into hexane using two modes of collection; hourly collection over four hours and a continuous collection over four hours. The five samples were analysed on GC-MS fitted with HP 5 MS capillary column using a temperature program; 70 degrees (3min) increased at 5 deg. / min. to 220 deg. (2min.) further increased at 6 deg. / min to 240 deg. (8min.). The major constituents are cis-vaccenic acid (11.3-44.6 %), 6-octadecenoic acid (Z)-(6.8-27.2 %), n-hexadecanoic acid (0.4-26.6 %), 9-octadecenoic acid (Z)-, methyl ester (0.4-29.9 %), 9-octadecenal (7.9-28.2 %), naphthalene (1.3-17.3 %), 9, 12-octadecadienoic acid (Z,Z)-, methyl ester (0-7.4 %), 9-octadecenoic acid (Z)-2-hydroxy (0-7.1 %), octadecanoic acid, methyl ester (0-3.3 %) and docosanoic acid, methyl ester (0-2.8 %). The minor constituents include C10-C18 alkanes as well as methylated alkane and benzenes. The essential oil samples are very rich in unsaturated fatty acids and derivatives and hence should exhibit antimicrobial and anti-inflammatory properties. They can also serve as scavengers for free radicals and thus eliminate uncontrolled cell proliferation and may thus demonstrate anti-tumor properties.
The fresh leaves of *Pseudocedrela kotschyi*, a medicinal plant used in the management of sickle cell disease, SCD, were processed for hydrodistillation to obtain the essential oil which was collected in two modes; hourly collection over four hours and a continuous collection for four hours. The five samples were analyzed on GC-MS fitted with HP 5 MS capillary column using a temperature program; 75°C (2 min.) increased at 4°C / min. to 140°C (1 min.) and further increased at 3.5°C / min. to 220°C (2 min.) and then increased at 6°C / min to 250°C (1 min.). The major constituents are 1,6,10-dodecatrien-3-ol, 3,7,11-trimethyl (7.8-13.2%), benzoic acid, hexyl ester (7.7-17.7%), 3-hexene-1-ol, benzoate (0.8-11.4%), nerolidol 2 (0-7.9%), α-farnesene (0.3-5.6%). Other constituents include τ-muurolol (0.5-1.3%), copaene (0-1.8%), caryophyllene (1.7-3.5%), α-caryophyllene (1.5-3.1%), hexanoic acid, hexyl ester (0-0.7%), α-cadinol (0-2.7%) and small quantities of naphthalene including its dihydro, octahydro and decahydro derivatives, long chain alkanes, isophytol, phytol, benzyl benzoate and n-hexadecanoic acid. The anti-inflammatory components, namely phytol and caryophyllene may relieve pain in SCD, n-hexadecanoic acid can function as anti-microbial while hexanoic acid, hexyl ester formed from short chain carboxylic acid may have anti-sickling effect.

Keywords: Analysis, Chromatography, Gas Chromatography/Mass Spectrometry, Natural Products
BIOCORIN (New Biocoating for Corrosion Inhibition in Metal Surfaces) is a project funded by the European Union under the activities of the Seventh Framework Programme (FP 7-ENVIRONMENT). Corrosion is a global problem worldwide and the direct economic loss due to metal corrosion of infrastructures and equipments is estimated to exceed €1.32 trillion per year, which accounts for 3 to 4% of the Gross Domestic Product of industrialized countries. Among corrosion types, Microbial Induced Corrosion (MIC) caused by fouling is involved in at least 10% of the corrosion of structures, and up to 50% in the case of subterranean pipes.

BIOCORIN project, aims to develop a green alternative to the coatings and solutions used up to date for MIC corrosion control. Some of the results of this project are presented here, with the identification of several relevant antifouling compounds secreted by environmentally isolated anti-MIC strains of microorganisms via a metabolomic approach.

Metabolites were extracted from 100 ml cultures incubated in Terrific Broth (TB) at 30°C for 24 h of three anti-MIC bacterial strains grown in the presence (250 l or 2 ml) and absence of a pool of MIC extracts used as inducers originated from continental Mediterranean climate, Atlantic climate, Mediterranean climate and Oceanic climate. Each culture broth was recovered and analyzed by means of LC-MS and GC-MS (Acquity UPLC - SYNAPT G2 HDMS system QToF with ESI injection and a Thermo Scientific Focus GC - DSQ II respectively). The metabolomics analysis pointed out the differential expression of 2 compounds by GC-MS and more than 10 compounds for LC-MS (positive mode) relevant to the corrosion process.

Keywords: Metabonomics, Environmental, Liquid Chromatography, Metabolomics
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Liquid Chromatography/Mass Spectrometry
**Abstract Text**

[b]Introduction[/b]
So far the analysis of intact molecules via Matrix-assisted-laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) above a molecular weight of 50,000 Da remains restricted due to the detector limitations, i.e. typically electron multipliers. To eliminate these limitations we developed a new ion detector, based upon thermo-mechanical effects suspended silicon nanomembranes.

[b]Methods and Materials[/b]
The novel detector consists of nanomembranes (trilayer of Al/Si$_3$N$_4$/Al), an extraction gate, a microchannel plate, and an anode. This assembly is placed at the end of the flight tube of a commercial MALDI-TOF-MS (Voyager-DE STR; Perseptive Biosystems). Detector performance was tested by analyzing intact proteins of diverse molecular weight via MALDI-TOF-MS.

[b]Results[/b]
Using the nanomembrane detector the singly charged ions of Insulin (5,729 Da), BSA (66,429 Da), IgG (150,000 Da), IgM (983,000 Da) were detected even with protein solutions in the nanomolar range. Additionally the singly charged ion of the IgG heavy chain (HC) was detected. The average resolution determined for IgM was $m/\Delta m = 250$, 129 for IgG and 95 for BSA. Thus, the FWHM mass resolution of the detector increased with the mass of the analytes.

[b]Discussion/Conclusion[/b]
Our results demonstrate that MALDI-TOF equipped with a silicon nanomembrane detector allows the analysis of intact protein mixtures over a broad mass range. This technology surpasses the limitation of current ion detectors towards the megadalton range providing high resolution at room temperature. The results indicate that peak height and the frequency of the nanomembrane response, are independent of the $m/z$ of the impinging ions.

**Keywords:** Bioanalytical, Biotechnology, Detector, Mass Spectrometry

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
H-bonding of tryptophan (Trp) stabilizes proteins and can aid protein translocation through cell membranes, and Trp oxidation in proteins, which can proceed by radical reactions, affects protein structure and function. H-bonding and radical reactions of Trp were investigated exploiting the electrochemical (EC) and soft ionization capabilities of electrospray ionization (ESI) using an on-line EC/ESI- mass spectrometry (MS) system. Trp oxidation products, i.e., hydroxy- and dihydroxy-Trp, [(OH+Trp)+H]+ (m/z 221) and [(2OH+Trp)+H]+ (m/z 237), respectively, were detected in the ESI mass spectra of 50 µM Trp in ESI carrier solution of 50/49/1 vol%, MeOH/H2O/HAc, pH 4.3, at 50 µL/h. In addition, covalent Trp dimers, assigned to [2Trp-6H+H]+ (m/z 403) and [2Trp-2H+H]+ (m/z 407), were detected. The formation of covalent dimers presumably involves Trp radicals generated during ESI. In addition, corresponding covalent hydroxy-Trp dimers [(OH+Trp+Trp)+H]+ (m/z 419 and 423) were detected indicating that both oxidation and radical formation occurs during ESI. Trp dimers, [2Trp-4H+H]+ (m/z 405) and [2Trp+H]+ (m/z 409), and the corresponding hydroxyl-Trp dimers (m/z 421 and 425), were assigned to Trp proton bound dimers. The structure of proton bound Trp dimer ion, [2Trp+H]+ (m/z 409), was verified by infrared multiple photon dissociation spectroscopy (IRMPD). When 2 V was applied to the EC cell in an on-line EC/ESI-MS, the intensity of Trp oxidation product and dimer ions increased. EC/ESI-MS thus offers an alternative technique for studying oxidation, radical reactions and hydrogen bonding of Trp.

Keywords: Bioanalytical, Electrochemistry, Mass Spectrometry, On-line
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Monoclonal antibodies (mAb’s) are the fastest growing class of therapeutics. Current best practices require qualification and eventual validation of in-house reference standards for mAb therapeutics to be used for quality testing of subsequent lots. During the drug development stages of a product lifecycle, the in-house reference standard must co-evolve with process changes affecting quality attributes as well as analytical test methods. Qualification of test methods as well as in-house reference standards would be greatly supplemented by a widely available representative test material. This presentation will discuss an ongoing effort for establishing an IgG1k National Reference Material to be used as a system suitability tool expected to more firmly underpin regulatory decisions and facilitate the development of originator and follow-on biologics. The RM is intended for a variety of uses including, but not necessarily limited to: system suitability tests, establishing method or instrument performance and variability, comparing changing analytical test methods, assisting in method qualification, etc. The NIST mAb characterization will be discussed in association with potential utility during an originator or follow on manufacturer’s process/product development process. Results from an ongoing round robin characterization study and book project including over 60 contributors from industry, academia, and regulatory agencies will also be discussed.

Keywords: Biomedical, Biopharmaceutical, Liquid Chromatography, Mass Spectrometry
Application Code: Biomedical
Methodology Code: Mass Spectrometry
The direct analysis of intact proteins via mass spectrometry offers compelling advantages due to the direct and unambiguous identification and characterization of protein sequences it provides. The inability to efficiently analyze proteins in the ‘middle mass range’ (30-80 kDa), in a robust fashion has limited the adoption of these “top-down” methods. Largely a result of poor liquid chromatographic performance, the limitations in this mass range may be addressed by alternative separations that replace chromatography. Herein, the short migration times of CZE-ESI-MS/MS have been extended to size-sorted whole proteins in complex mixtures from Pseudomonas aeruginosa PA01.

Protein fractionation was performed in GELFREE 8100 System. The fractions were then suspended in 5 [micro]L of 0.1% formic acid after SDS removal. The separation capillary (id 50 [micro]m, od 150 [micro]m, length 30.0 cm) was coated with poly-arginine, then filled with 0.1% formic acid. 240 nL of stacking buffer (60% isopropanol, 0.8% formic acid) and 80 nL of each fraction were injected hydrodynamically in sequence. The injection end was immersed in 0.4% formic acid, with -2 kV applied for 30 minutes. Each fraction was analyzed in triplicate. Data were collected in Q-Exactive mass spectrometer and analyzed with ProSightPC 3.0.

The CZE-ESI-MS system separated a mixture of standard proteins and achieved peak widths at 50% peak height as narrow as 8 s for trypsinogen, 12 s for myoglobin, carbonic anhydrase and superoxide dismutase, and 16 s for ubiquitin.

This work identified 65 proteins from 10 fractions with 30 of the identified proteins above 30 kDa. The intact mass of 16 proteins (30-50 kDa) could be manually validated by MS1 data. The peak widths at 50% peak height of 12 proteins were as narrow as 24 to 57 s, while two abundant proteins were 84 and 78 s.

These results suggest that CZE-ESI-MS/MS is capable of identifying proteins in the middle mass range in top-down proteomics.

Keywords: Capillary Electrophoresis, Mass Spectrometry, Protein, Proteomics
Application Code: Genomics, Proteomics and Other ‘Omics
Methodology Code: Mass Spectrometry
Identification of the Sulfone Functionality in Protonated Analytes via Ion/Molecule Reactions in a Linear Quadrupole Ion Trap Mass Spectrometer

A mass spectrometric method is presented for the rapid identification of compounds that contain the sulfone functional group. This method is based on selective gas-phase ion/molecule reaction of protonated sulfone analytes by trimethyl borate (TMB) that yields diagnostic product ion adduct-Me2O at high reaction efficiency. A variety of compounds with different functional groups, such as sulfoxide, hydroxylamine, N-oxide, aniline, phenol, aliphatic amine and alcohol are examined to probe the selectivity of this reaction. The result showed all the other compounds react very slowly or not at all with TMB, and none of them give the adduct-Me2O product. This method also successfully distinguishes anti-inflammatory drug Sulindac and its metabolite Sulindac sulfone, indicating the potential use of this method in the analysis of sulfone containing drug and drug metabolite.

Keywords: Analysis, Drug Discovery, Method Development, Organic Mass Spectrometry
Application Code: Pharmaceutical
Methodology Code: Mass Spectrometry
Quantitative and effective techniques for blood sampling and analysis are required to meet the growing needs of biomedical and bioanalytical research. Traditional blood draws are inherently disadvantaged because of the amount of blood typically withdrawn from the patient. Dried matrix spots (DMS) of blood overcome this shortfall because only finger or heel pricks are required. DMS greatly reduces the volume of collected sample, thus allowing easier procurement from even infants. 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 pressing importance to bioanalytical research. This 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. MALDI as a quantitative method will be an alternative ionization method. By coupling MALDI with QTOF mass spectrometry in conjunction with Isotope and Speciated Isotope Dilution Mass Spectrometry enables quantification and the figures of merit for accuracy and precision will be discussed.

**Keywords:** Bioanalytical, Laser Desorption, Mass Spectrometry, Quantitative

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
Malignant gliomas are among the most fatal cancers. Even with aggressive multimodal therapy, patient survival is measured in months. Our coworkers found intratumoral injection of CpG conjugated with single-walled carbon nanotubes (SWCNT-CpG) was far superior to free CpG therapy and eradicated intracranial gliomas in more than 60% of mice. This effect was unique to the SWCNTs; similarly functionalized gold, iron oxide or PLGA NPs carrying CpG were ineffective at activating NF-κB in vitro and had no additional in vivo anti-tumor efficacy over free CpG.

We hypothesize that, for nano-CpG to work effectively, an appropriate protein corona is needed to not only improve cell internalization, but also modulate the cellular response to the carrier itself, which may affect the efficacy of the carried drug.

In this study, we developed a method coupling centrifugation with LC-MSMS to reveal the composition of protein corona on CpG-SWCNT in macrophage cell lysate. The surface coating of CpG-SWCNT is optimized as 6:1 Lipid-PEG-NH2:Lipid-PEG-CpG to achieve good stability and anti-tumor efficacy while SWCNT coated with only Lipid-PEG-NH2 serves as control. Surface-modified SWCNTs were incubated with macrophage cell lysate, and then went though a few circles of washes to remove unbound proteins by centrifugation. Tryptic digestion was applied to the protein corona covered SWCNTs. Finally, the obtained peptides were analyzed by LC-MSMS. 28 proteins were uniquely identified in the protein corona of CpG-SWCNT. Among them, we found Tyrosine-protein kinase BTK, which is a key component in the signaling pathway linking TLR8 and TLR9 to NF-κB. The TLR9 is reported to be the receptor for CpG ligand and the activation of NF-κB is essential for triggering anti-tumor immunotherapy. The affinity and stability of tyrosine-protein kinase BTK in the protein corona should have a great contribution to the tumor-killing effect of CpG-conjugated nanomaterials.

**Keywords:** Liquid Chromatography/Mass Spectroscopy, Nanotechnology, Protein, Proteomics

**Application Code:** Biomedical

**Methodology Code:** Mass Spectrometry
The specificity of an enzyme is influenced both by the structure of the enzyme’s active site and the presence of adaptor domains that localize the enzyme with respect to substrates. The interconnected properties of the catalytic and adaptor domains influence the overall specificity of the enzyme, and yet, the evolution of this interdependency remains poorly understood. Since the main purpose of an adaptor domain is to localize the protein at or near its substrate, traditional solution based assays fail to fully capture the influence of adaptor domains on enzyme specificity and kinetics. A surface based assay like Self-Assembled Monolayer assisted MALDI mass spectrometry, SAMDI, is better suited to monitor kinetic changes that occur in an enzyme when adaptor domains are present or omitted. In this work, we studied two highly conserved PTPs with very different kinetic efficiency, PTP1B an adaptor-less protein and SHP-1, an enzyme containing SH2 adaptor domains. We identified five specific amino acids that are different in the catalytic domain of the SHP-1, compared to the PTP1B, and mutated the catalytic domain of the PTP1B to reflect these differences. PTP activity was analyzed using SAMDI on a library of 361 peptides containing a phosphorylated tyrosine. The peptide library allowed changes in kinetic rates to be analyzed, and also, alterations in substrate specificity. Overall, this work illustrates the evolutionary relationship between catalytic activity and adaptor domains.

**Keywords:** Bioanalytical, Enzyme Assays, Mass Spectrometry

**Application Code:** Bioanalytical

**Methodology Code:** Mass Spectrometry
D-amino acids play an important role in the biological function of certain peptides. D-Ser and D-Asp have both been detected in animal tissues where it is hypothesized that spontaneous racemization occurs with aging. However, the presence of D-amino acids is difficult to directly detect by mass spectrometry since no mass shift is involved. Also separating epimers by liquid chromatography (LC) can be difficult since the conformational change between epimers is not significant. It has been demonstrated that Radical directed dissociation (RDD) provides significantly better chiral discrimination of peptide epimers than normal collision induced dissociation (CID). In RDD, peptides are covalently labeled by chromophores that include a carbon iodine bond. UV irradiation of the labeled peptide homolytically cleaves the C-I bond and yields a radical, followed by radical migration and dissociation. The fragmentation is dominated by radical dissociation chemistry and primarily occurs in the vicinity of the radical, which differs from CID. In this study, RDD coupled with HPLC is demonstrated as a powerful method which can detect peptide epimers in a complex mixture. Three types of crystallin protein was extracted from sheep eye lens and digested with trypsin. After covalent labeling with 4-iodo-benzoic acid and LC-MS, the sequence coverage is around 75% and nearly ten D-amino acid containing peptide was detected by RDD. The covalent modification for introducing the radical can also improve the separation of peptide isomers.

Keywords: Amino Acids, Chiral, Liquid Chromatography/Mass Spectroscopy, Proteomics
Application Code: Genomics, Proteomics and Other 'Oomics
Methodology Code: Mass Spectrometry
Mass Spectroscopy: Bioanalytical and 'Omics

High-Resolution Enabled 10-plex DiLeu Isobaric Tagging Reagents for Mass Spectrometry-Based Relative Quantitation

Multiplexed isobaric labeling has become a powerful method for mass spectrometry-based relative quantitation of proteins and endogenous peptides, and several commercially available products are available for such experiments. However, the high cost of these reagents is often prohibitive. We have developed our own set of multiplexed isobaric labeling reagents, DiLeu, featuring N,N-dimethylated leucines as reporters that can be readily synthesized using commercially available chemicals at greatly reduced cost. The DiLeu reagent resembles the general structure of other isobaric labeling reagents in that it contains a reporter group, balance group, and amine-reactive group for targeting the N-terminus and Lysine side chain of a peptide. Each incorporated label results in a mass shift of 145.1 Da, and intense reporter ions at m/z 115.1, 116.1, 117.1, and 118.1 are observed for the pooled samples upon tandem-mass fragmentation. An increase in multiplexing rate can be achieved through selective incorporation of 15N, 13C, and deuterium stable isotopes in the reporter structure to create six additional isotopologues that that differ from the existing four reporters by 6-12 mDa. We show that these reporters can be baseline-resolved in high-resolution Orbitrap Elite higher-energy collisional induced dissociation spectra to enable 10-plex quantitation without compromising quantitative accuracy and dynamic range. To demonstrate the reagents’ quantitative performance, BSA tryptic digest was labeled with each of the ten DiLeu reagents, combined in 1:1:1:1:1:1:1:1:1:1 and 16:8:4:2:1:1:2:4:8:16 ratios, and cleaned up via strong cation exchange pipette tips. LC-MS/MS analysis was performed on an Orbitrap Elite system to highlight the utility of high-resolution enabled multiplex quantitation.

Keywords: Mass Spectrometry, Quantitative, Tandem Mass Spec
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Mass Spectrometry
Mass Spectroscopy: Bioanalytical and 'Omics

Direct MALDI Imaging of Glycosphingolipids (GSL) in Brain Tissue of Mouse Models of Lysosomal Storage Disorders

GSLs are highly expressed in the central nervous system (CNS) and are found in neuronal cells. These lipids are involved in biological processes vital for normal function such as: cell-to-cell interactions and the modulation of protein activity in the plasma membrane. Aberrations in GSL expression causes a class of disease known as lysosomal storage disorders (LSDs) which result in the accumulation of glycolipids at pathological levels causing neurological dysfunction. There is a growing interest in the study of GSL metabolism in mouse models of LSDs. The use of imaging techniques such as MALDI to study the spatial location of gangliosides and other GSLs with allow us to gain a better understanding of lipid expression and their functions in disease progression. Preliminary results indicate there are clear variations in sphingolipid in brain tissue of Niemann-Pick C Disease mouse model.

Keywords: Bioanalytical, Data Analysis, Imaging, Mass Spectrometry

Bioanalytical

Mass Spectrometry
Cleaning validation is a fundamental part of current good manufacturing practices in any pharmaceutical industry. Nowadays, several pharmacologically potent pharmaceuticals are manufactured in same production area. Carefully designed cleaning procedures and its validation can ensure that residues of the API will not carry over and cross contaminate the subsequent product. The cleaning procedures must be validated and the methods to determine trace amounts of drugs have therefore to be considered with special attention. A rapid and sensitive reversed-phase high performance liquid chromatographic method to determine trace levels of reserpine in rinse and swab samples collected from the equipment surfaces has been developed and found to be accurate and precise. Reserpine is a low dose compound and is considered to be the worst case regarding its solubility. Residues were determined by high-performance liquid chromatography on a C18 BDS thermohypersil 25 cm × 4.6 mm, 5 µm column at 25°C in the isocratic mode with 1:1 mixture of acetonitrile and aqueous ammonium chloride solution (1 : 100 w/v), at pH 5.6 as the mobile phase. UV detection was performed at 218 nm. A systematic protocol for setting the allowed residual limit and validating the analytical method regarding specificity, limit of detection, recovery, precision, and stability of standard and sample solutions was utilized. In addition, the stability of the swab samples with analyte was evaluated to determine the allowable time interval between sampling equipment surfaces and extraction of the analyte with sample solvent. The method was validated over a concentration range of 24 - 320 ng/ml. The limits of detection and quantitation were evaluated to be 8 and 24 ng/ml, respectively. The stability of reserpine at different steps of the sampling procedure and the precision of the swabbing procedure were also investigated.

Source of Funding:
This work was supported by University of Alexandria, Egypt.

Keywords: HPLC
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Selectivity is the most powerful term in the resolution equation. Understanding which parameters affect selectivity is helpful for rapid method development. In this poster, we investigate the influence of stationary phase design on selectivity with a novel, polar embedded stationary phase: ACE C18-Amide. This new stationary phase has been specifically designed to minimise phase bleed, be stable at low and intermediate eluent pH values and have enhanced hydrophobicity whilst maintaining polar retention and phenolic resolution properties. The impact of organic modifier choice and eluent pH on selectivity values was also determined and highlighted the versatility of the C18-Amide phase. A variety of polar analyte separations including catecholamines, resorcinols, vanillins, wine acids and steroids were developed to explore retention, elution order and chromatographic selectivity with different conditions.

**Keywords:** Chromatography, HPLC, Liquid Chromatography, Method Development

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography
The root cause investigation of particle contamination in pharmaceutical production requires reliable chemical identification techniques. It is standard practice in many particle characterization laboratories to use several instruments to perform material identification techniques such as: SEM/EDS, FT-IR or Raman microscopy, resulting in the transfer of the micro-object from one instrument to another. As these particles are so small it is hard and time consuming to find them on a micrometer scale each time and can lead to errors. Besides manual obstacles, each instrument works with its own chemometrical system and produces independent data. The pharmaceutical industry works in a regulated environment. 21 CFR Part 11 compliance is a strict rule for the collection of the raw data. State of the art equipment combines multiple techniques in one system and produces traceable and compliant data. This data enables chemical characterization of the micro-particles and matches the material with those used in the manufacturing of the pharmaceutical. This unit cancels the need for sample transfer between machines, analysis does not require spectroscopic expertise (as it is automated), is user friendly, and all results are compared to a built-in matrix database, and corresponding reports are generated. Once the spectral fingerprints are matched to the database, results can be used to determine the particles origin and compare samples. Raman Spectroscopy and laser-induced breakdown spectroscopy (LIBS) are both significant contributors to the delivery for the ‘whole picture’ of a microscope. Both techniques analyze micro-samples using lasers. Therefore, the system delivers multi-elemental composition analysis whereas Raman yields information about the molecular composition. The combination in one product delivers a unique tool for the quick determination of micro-particle samples, especially foreign particulate matter investigations.

Keywords: Atomic Spectroscopy, Materials Characterization, Molecular Spectroscopy, Raman
Application Code: Quality/QA/QC
Methodology Code: Pharmaceutical
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: Analysis, Atomic Spectroscopy, Biopharmaceutical, Molecular Spectroscopy

Application Code: Pharmaceutical
Methodology Code: Molecular Spectroscopy
Abstract Text

We report a new method to separate guanidine compounds in water that uses macrocycle-based ion chromatography (IC) with pulsed amperometric detection (PAD). Ion chromatographic columns were packed with a glutamic acid based cavitands (GAU) to accomplish analyte separation. The glutamic acid substituted resorcinarene cavitands were absorbed on divinylbenzene macroporous resin. Guanidine (G), 1,1-dimethylguanide (DMG), 1-(4-aminoguanidobutane (agmatine, AGM), 2-cyano-1-methyl-3-(2-[(5-methyl-1H-imidazol-4-yl)methylthio]ethyl)guanidine (cimetidine, CIM) and 1,3-ditolylguanidine (DTG) were separated in water with gradient elution by methanesulfonic acid (MSA) eluent. The separation was compared to that obtained with the commercial IonPac CS14 column, which required acetonitrile in the eluent. Limits of detection of the compounds were measured with pulsed amperometric and conductivity (CD) detectors.
BET Surface area analysis of lyophilized products has, to this point, required sample manipulation and necessitated techniques that are destructive to the cake prior to testing. Due to the nature of lyophilization processes, destruction of the cake and subsequent removal of the sample from its vial is required. This type of traditional sample preparation technique for BET surface area testing introduces error resulting in lack of repeatability and robustness of the analysis and possible misrepresentation of the true surface area of the sample. Because the sample preparation is destructive additional testing and analysis of the cake was not possible.

Analysis-to-analysis variability stemming from non-standardized preparation techniques can lead to artificially high surface areas. In this study, we have developed a technique that will allow for surface area measurement of intact lyophilized cakes that is both repeatable and robust and will allow for more repeatable measurement of this important control parameter in lyophilized product characterization. We have developed a special lyophilization vial holder apparatus that attaches to the Micromeritics ASAP 2420 Surface Area and Porosity Tester that will allow for non-destructive, intact lyophilized cake testing of surface area. In this preliminary study, we have found that surface area measurements of intact lyophilized cakes are approximately ¼ - ½ the surface area of the same samples that have been destructively prepared. Further development of this technique will lead to an invaluable characterization technique for lyophilized products during product development, scale-up, and as an implementable PAT tool for control of this critical process parameter.

Keywords: Materials Characterization, Pharmaceutical, Sample Preparation, Surface Analysis
Application Code: Pharmaceutical
Methodology Code: Physical Measurements
In the world of separation science the pharmaceutical industry has many requirements, high throughput screening (HTS) of potential NDA candidates, highly selective methodology for separation of parent API from any potential interfering metabolites, chiral and genotoxic impurities. Sensitivity of analyte analysis to provide high LOD & LOQ limits to be achieved leading to robust qualitative/quantifiable results is also critical.

The introduction of core-shell particles into the separation sciences has lead to potential improvements in all of the requirements above.

In this work we look at the ability to achieve fast, selective, sensitive LC methods with these core-shell products. We analyse a range of pharmaceutical drugs in order to highlight the improvements seen.

### Keywords:
- Chromatography, High Throughput Chemical Analysis, HPLC, Pharmaceutical

### Application Code:
- Pharmaceutical

### Methodology Code:
- Liquid Chromatography
Use of Additives for Improving Chromatographic Analysis

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 or the new Titan particles represent key developments that enable the full performance of a UHPLC system with a maximum of sensitivity and chromatographic efficiency. Unfortunately these enhancements can be undermined by the use of unsuitable solvents. Examples, e.g. vitamin D show the influence of solvent quality especially on the MS detection. A suppression effect occur although the matrix is well separated from the analyte.
The development of chromatographic methods for pharmaceutical products can be challenging for many reasons. These challenges can include changes in selectivity under different elution conditions, poor or no UV absorbance, or similar UV profiles between components among others. To monitor changes in selectivity practitioners often utilize standards, analyzed separately, under each of the separation conditions to efficiently track components. For samples which contain components that exhibit poor or no UV absorbance, practitioners may need to utilize several modes of detection such as UV and evaporative light scattering (ELS).

We will present the development of a method for analyzing the active pharmaceutical ingredient memantine and its associated metabolites. Memantine is commonly used for the treatment of dementia which is often associated with the onset of Alzheimer's disease. Each of these components exhibits no UV absorbance, and currently their detection is accomplished with ELS or by post column derivitization to allow indirect UV detection. While these approaches have shown utility, they lack the specificity often desired in assays.

Our presentation will demonstrate the use of a robust mass detector for efficiently tracking these components during the method development process. We will demonstrate the linearity, reproducibility, and specificity achievable with mass detection, which would allow this technology to be deployed for routine assays. We will also discuss how an easy to use mass detector can provide reliable data to guide decision making during the development of chromatographic methods and their deployment for routine use.

Keywords: Chromatography, Detector, Mass Spectrometry, Pharmaceutical
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Once a chemical hit is found through a library screening process and verified, optimization of the synthetic route and the compounds desired properties takes place. This step involves an iterative process of synthesis and reactivity measurement of the new compounds to further develop drug candidates into the lead phase. Reactions may proceed along a course of minutes, hours, or days. During this process, chemists need to know as soon as possible if their syntheses are proceeding as desired. This means utilizing measurement capabilities that require minimal sample preparation with a fast response giving low detection limits. In addition, it is often necessary to monitor multiple parameters or parallel reactions simultaneously.

In this presentation we present the use of Ultra Performance Chromatography coupled with robust mass detection for monitoring the synthesis of several active pharmaceutical ingredients. Our results will include an optimized screening approach to monitor chiral and achiral components with a single chromatographic system when coupled with a mass detector. In addition, we will discuss workflows that demonstrate the utility of mass detection during analysis, purification, and post-purification analysis that The benefits of these approach will show improvements in analysis time via a streamlined decision making process as well.

Keywords: Drug Discovery, Mass Spectrometry, Pharmaceutical, SFC
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography/Mass Spectrometry
Abstract Text
FT-microwave spectrum of cyclopentylamine, c-C5H9NH2, has been recorded, and the transitions have been assigned for the most stable conformer, and the rotational constants have been determined. The vibrational spectra have been investigated in detail with the recording of the Raman spectra (3800-50 cm\(^{-1}\)) of the liquid and the infrared spectra (3700-200 cm\(^{-1}\)) of the gas. Additionally, the variable temperature (-60 to -100°C) Raman spectra of the sample dissolved in liquefied xenon with complete spectra record at -60 and -100°C. In the region where significant differences were observed in the relative band intensities the spectra were recorded at every ten degree intervals. From these data two conformers have been identified with one the trans-axial form and the other trans-equatorial conformer. The axial and equatorial orientations refer to the ring atoms whereas the trans and gauche orientations refer to the position of the NH\(_2\) group. The four possible conformers have been identified and their relative stabilities obtained with enthalpy difference relative to the most stable conformer. The conformational stabilities have been predicted from ab initio calculations utilizing several different basis sets up to aug-cc-pVTZ from both MP2(full) and density functional theory calculations by the B3LYP method. Vibrational assignments have been provided for the observed bands for all four conformers which are predicted by MP2(full)/6-31G(d) ab initio calculations to predict harmonic force constants, wavenumbers, infrared intensities, Raman activities and depolarization ratios for all conformers.

Keywords: Infrared and Raman, Raman, Spectroscopy, Vibrational Spectroscopy
Application Code: Pharmaceutical
Methodology Code: Vibrational Spectroscopy
The USP <467> method is widely used for the identification and quantification of residual solvents in pharmaceutical products. It applies to all compendia drug substances excipients and products. Levels of residual solvents need to be monitored and controlled for a number of reasons including human and environmental safety. This is why the USP <467> method requirements are designed to ensure that the potential presence of residual solvents is reduced to relatively low concentrations.

In particular the USP <467> describes the use of static headspace sampler coupled with a GC-FID system in order to recognize and quantify organic volatile impurities. This paper shows the benefits obtained using the Headspace technique coupled with a GC/TOF-MS system capable of analyzing all classes of compounds in a single analysis reducing drastically the time analysis. Data will be reported including chromatographic parameters, qualitative and quantitative determinations. In addition, alternative approaches to conventional analysis will be provided.
Diastereomer separation in Tenofovir prodrug is the difficulty of the drug purification process. To establish a HPLC craft for separating diastereomers in Tenofovir prodrug, we use NP-HPLC combining RP-HPLC method: Venusil XBP C18(2) (30*250mm 10 μm 100Å) chromatographic column, acetonitrile-water RP-HPLC system, followed by Venusil XBP Silica (30*250mm 10 μm 100Å) chromatographic column and normal hexane-ethanol NP-HPLC system. By this two-step purification, we obtain the products with purity higher than 98% (by area normalization), and the recovery rate is higher than 65%. This craft is accurate, easy to operate, and with good reproducibility.

**Keywords:** Drugs, Pharmaceutical, Prep Chromatography

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography
Antibody drug conjugates (ADCs) have special therapeutic benefits as they combine the selectivity of monoclonal antibodies with a highly potent drug to attack cancer cells. For the manufacture of ADCs, a reactive linker is attached to the small molecule drug to make a reactive linker drug intermediate. The linker then reacts with the protein to attach the drug and antibody together to form the drug substance. As the linker drug is designed to be reactive with proteins, it presents challenges for both long term stability and method development for the reactive linker drug.

In this study, the ADC linker drug contains an amine reactive tetrafluorophenyl (TFP) ester that conjugates the drug to lysine residues on the carrier antibody. The HPLC method development for this reactive linker drug is very challenging as the TFP ester readily undergoes hydrolysis and an internal succimide cyclization in aqueous mobile phase on the column. This results in the generation of artifact peaks in the chromatograms and delivers the wrong impurity content results. In order to develop a valid assay and impurity method for this reactive linker drug, optimization of the critical chromatography conditions to minimize the degree of on-column degradation must be performed. Chemical kinetics is employed to study the degradation rates of the linker drug in different solution pH and temperatures. Reaction rates were used to guide the selection the optimum mobile phase pH, column temperature and column type to minimize the on-column degradation of the linker drug. We also calculated the actual amount of on-column degradation to demonstrate the effectiveness and accuracy of the method. This chemical kinetics strategy for HPLC method development is much more efficient than conventional try and error approach, especially for reactive compounds and can be applied to other reactive ADC linker drugs as well.
Sampling and Sample Preparation

Development of Novel Passive Air Sampler for Simultaneous Determination of NO and NO$_2$ Employing Ceria/quartz Fiber Filter

Passive air sampler has been widely used for indoor, outdoor and personal exposure monitoring of various air pollutants including NO and NO$_2$. PTIO (2-phenyl-4,4,5,5-tetramethylimidazoline-3-oxide-1-oxyl) has been previously employed as an oxidizing agent of NO for the collection as nitrite in a trapping filter of the passive sampler. However, analytical uncertainty caused by PTIO was claimed, because it is labile to light and heat, and interferes the colorimetric determinations by a reaction with color reagents (sulfanilamide/NEDA). Then, this study aimed to alter the PTIO employing ceria as a novel trapping material of NO. A quartz fiber filter was immersed with aqueous cerium acetate solution, dried in an oven and then calcinated to form ceria on the fibers. The ceria filter was subsequently impregnated with triethanolamine and served for the trapping filter of both NO and NO$_2$. Sampling performance was compared with the previous PTIO-based filter by exposing the filters in air for 24 hours. Collection amount of NO, found as nitrite in water extract, was determined by both ion chromatography and sulfanilamide/NEDA method. The amount of NO by ceria-based filter equaled to those by PTIO filter with higher repeatability and further conversion to nitrate was negligible. Then, the ceria filter was practically applied to Oxford type passive sampler and the sampling rate was successfully determined by repeated exposure tests.

Keywords: Environmental Analysis, Environmental/Air, Sampling
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Sampling and Sample Preparation

Cloud Point Extraction of Metal Oxide (TiO$_2$ and ZnO) Nanoparticles in Water Samples Identified by Raman Spectroscopy and Quantified by Atomic Absorption Spectroscopy

Engineered nanoparticles have been shown to affect the growth of living organisms in freshwater such as lakes and rivers. It is important to acquire a suitable technique to identify nanoparticles and quantify them. Raman scattering offers the advantage of analyzing micro-scale nanoparticle species which is the maximum amount available in aquatic environment. In this project, cloud point extraction (CPE) with Raman spectroscopy and atomic absorption spectroscopy was applied to the analysis of titanium dioxide nanoparticles (TiO$_2$ NPs) and zinc oxide nanoparticles (ZnO NPs) in water and environmental water samples. The non-ionic surfactant Triton X-114 was used to extract the metal oxide nanoparticles from water. The sample was centrifuged to collect the surfactant, followed by Raman spectroscopy of the surfactant phase. NPs were hydrolyzed in concentrated HCl, and then diluted to give samples for flame AA. Several CPE factors such as surfactant concentration, pH, incubation temperature, and incubation time were optimized. Method performance was characterized in terms of the smallest concentration of NPs detected, by the selectivity over metal ions, and by the sensitivity of Raman spectroscopy to identify small quantities of NPs in mixtures.

Keywords: Environmental/Water, Extraction, Nanotechnology, Raman

Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Two new processes to pretreat blood samples have been developed. The treatments are based on a Fenton-like Advanced Oxidation Process (AOP) and use acid deactivation of the enzyme catalase in the blood. The first treatment is performed with a simple convection oven over a period of eight hours, while the second uses microwave irradiation for two minutes. The effect of pH on the process has been investigated and the degradation of whole blood has been compared with that of pure hemoglobin samples.
Sampling and Sample Preparation

Comparison of Sampling Methods for Identification of Process Tank Residues

After routine cleaning of process tanks, residues are sometimes observed on tank surfaces, appearing as faint whitish or dark stains that cannot be easily wiped off. It is desirable to identify the residue so that it can be removed without damaging the tank. Due to the small amount of residue on the surface, it is difficult to obtain enough sample for analysis. Currently, common sampling tools are cotton or polyester swabs. Swabs are poor sampling tools for tank residues because only a small amount is transferred to the swab, the sample is difficult to remove from the swab for analysis, and light-colored residues cannot be seen on the swab. We attempted to develop an alternative sampling method to obtain a larger sample which can be easily isolated for micro-analysis.

Cotton and polyester swabs were compared to razor blades, gloves, and other scraping tools fashioned from common laboratory and commercial items. The scraping tools were used to remove rust-colored and white residues from metal surfaces. Photographs are presented to demonstrate the relative amounts of sample obtained. Discussion is provided to describe the relative ease of removal of the samples for micro-analysis, possible contamination introduced, and suggestions for transporting the samples without loss or contamination.

The blade of a common polystyrene plastic knife was the most effective tool for removal of residues from metal surfaces. The knife blade removes a large amount of residue, does not introduce any contamination to the sample, does not damage the tank surface, and is inexpensive and simple to use. The knife blade with the collected sample can be transported in a 50mL plastic tube without loss of the sample. Dark-colored knives can be used for light-colored residues, so that the residue can be easily seen. The blade with the residue is examined under a stereomicroscope, where the sample can be easily removed with a needle for micro-analysis.

Keywords: Microscopy, Sample Preparation, Sampling, Small Samples
Application Code: General Interest
Methodology Code: Sampling and Sample Preparation
The use of Accelerated solvent extraction (ASE) is well established in the environmental, chemical and food analysis arena. The benefit of ASE is fast extraction of analytes relative to conventional extraction techniques such as soxhlet extraction. It is well known that presence of moisture in samples can interfere with all extraction techniques that are based on a partition technique. Since the analyte tends to partition or distribute between the organic extraction solvent and the aqueous fraction from the sample, the recoveries may not be optimal for some analytes particularly when they are polar.

In ASE, typically the sample is mixed with a dispersant and loaded into a cell followed by extraction with a suitable solvent. It is therefore desirable to dry the sample prior to extraction. Most protocols use an off-line drying process for a relatively wet sample and this may take several hours. Post extraction removal of the moisture is feasible by adding sodium sulfate that dissolves in the aqueous fraction, but in the process drives the analyte to the organic phase. This approach however is an added step and requires careful addition of the sodium sulfate.

Recently an AsePrep MAP polymer product was introduced for the in-line removal of moisture in ASE. The polymer is added with the sample and the dispersant and loaded into a cell followed by extraction with a suitable solvent. The benefit of this approach is inline moisture removal becomes feasible and there is no added time required for drying the sample or pursuing a post extraction removal of moisture by adding salts. The polymeric material can be used in both in-line and off-line applications. We will show here some real life example applications and demonstrate the utility of a moisture removal polymer.

Keywords: Accelerated Solvent Extraction, Environmental, Environmental/Soils, Sample Preparation
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Proteomics has become one of the foremost bio-analytical fields directed towards the discovery, detection and characterization of proteins. An integral part of this analytical process typically involves some level of sample pretreatment due to the size and complexity of proteins and the matrix components which can interfere with detection at low levels. A common pretreatment approach is digestion of a protein to characteristic peptide fragments prior to mass spectral analysis. Methodologies for subsequent extraction of these peptides from biological matrices can be time consuming and ineffective. This poster will introduce new resin based solid phase extraction column technology that eliminates column conditioning and equilibration steps to extract a mixture of peptides from plasma and serum prior to LC-MS-MS. A relative comparison was conducted for the new column technology for the Evolute ABN Express SPE resin compared on Evolute ABN. The optimized sample preparation method developed on the EVOLUE ABN sorbent was demonstrated for the extraction of a diverse mixture of peptides (Angiotensin II, Angiotensin fragment 1-7, Vasoactive Intestinal fragment 1-12, human Endorphin, Glycoprotein Myelin fragment, Helicobacter 2-20, Elafin, and ACTH 1-24) spiked into a plasma or serum matrix. Using the new column technology, this method can be further streamlined which eliminates the conditioning and equilibration steps. The peptides were pre-treated with 2% TFA, washed with nonopure water to clean up phospholipids and salts and extracted on the new column technology using a methanol: water solution (70:30). The extracted peptides were analysed by LC-MS-MS with no dry down step. Recoveries varied for each peptide ranging from 60% to 90% for both sorbent technologies. The recovery performance for the new column technology was just as or more efficient than the standard SPE assay without the extra steps.

**Keywords:** Biological Samples, Mass Spectrometry, Peptides, Sample Preparation

**Application Code:** Bioanalytical

**Methodology Code:** Sampling and Sample Preparation
New Graphitized Polymer Carbons and Carbon Molecular Sieves for Sample Preparation Applications

Spherical, high purity graphitized polymer carbons (GPC) and spherical, high purity carbon molecular sieves (CMS) have been synthesized for use in sample preparation processes. The use of new spherical GPC and CMS particles in packed bed systems and coated devices denotes a recent advancement in these technologies.

New spherical GPC particles have been synthesized which possess a wide range of textural properties and surface chemistries. For example, a 50um monodispersed GPC possessing a surface of 100m²/g has been effective in improving the adsorption and flow characteristics of a solid phase extraction (SPE) device for pesticide, insecticide and herbicide analyses. A second example is a family of these carbons, with surface areas ranging from one to two hundred meters per gram used for SPE applications. GPC particles with sub-10um particle sizes have also been bonded to glass, metal and plastic substrates using patented, proprietary adhesives for sample preparation applications.

New spherical CMS particles have also been synthesized for SPE analyses. For example, a CMS with a large microporous regime allowed for the preparation of coated surfaces for the extraction and subsequent analyses of small molecular sized, airborne contaminants. These applications focused also on a range of analytes from light gases to the semi-volatiles in aqueous environments. The surface chemistries of several 600um CMS particles have also been studied and applied to bulk extraction processes. CMS particles with sub-10um particle sizes have also been bonded to glass, metal and plastic substrates using patented, proprietary adhesives.

Nitrogen porosimetry, helium pycnometry, particle size analyses, scanning electron microscopy and autotitration techniques were used to study the carbons. Adsorbent capacities and reversible adsorption characteristics have been determined using the respective sample preparation processes.

Keywords: Absorption, Solid Phase Extraction
Application Code: Environmental
Methodology Code: Other (Specify)
The determination of protein concentration in a solution is a common necessity in a wide assortment of clinical, academic, and industrial laboratories. The Coomassie (Bradford) protein assay is a standard spectroscopic assay that involves pipetting samples with the dye Coomassie Brilliant Blue G-250 to generate the spectral shift from brown to blue with increasing protein concentration. Complete kits provide a full package of sample, dye, consumables, and instructions, yet the preparation of the standard curve along with unknown samples can still be time consuming to complete manually. Increasing workflow efficiency is the goal of many laboratories today, who are streamlining the manual pipetting steps to achieve a higher rate of result consistency and productivity. The core function of routine pipetting is shifting to simple automated liquid handlers capable of mimicking the manual process, and this application demonstrates the comparative results of automated liquid handling versus manual pipetting for the simple, yet common,
The mosquito Aedes aegypti is an international scourge responsible for spreading 50 to 100 million infections of dengue fever a year, as estimated by the National Institute of Health. Disease prevention requires a multi-pronged approach to limit transmission. This study proposes a method to break the reproductive cycle at the egg-laying stage. Small molecule oviposition attractants were tested in a high-throughput analysis with digital video identification. A 96-well plate was illuminated with infrared light and small molecule olfactory attractants were spotted. A pair of Microsoft Lifecam Studio USB cameras were connected to a Windows desktop with an Intel I7 processor. National Instruments software was used to control data acquisition and script a Bayesian model to identify objects tracked. Identified attractant mixtures were separated with gas chromatography, and detected with electroantennography and flame ionization detector simultaneously. Mosquitoes were injected with double stranded RNA to knockdown specific odorant binding proteins. This assay determines how wild type gravid female olfaction perception changes by correlating neuronal spiking with protein expression regulation.

**Keywords:** Bioanalytical, Monitoring, Sample & Data Management, Software

**Application Code:** Bioanalytical

**Methodology Code:** Sampling and Sample Preparation
Nicotine is of importance as the addictive chemical in tobacco and is also administered to aid smoking cessation. It is also a potential medication for several conditions including ulcerative colitis, Alzheimer’s and Parkinson’s diseases and is a useful probe drug for phenotyping cytochrome P450 2A6 (CYP2A6). Nicotine is rapidly broken down in the body with a half-life of approximately 2 hours. The major metabolite of nicotine is cotinine which is more stable with an approximate half-life of 20 hours, therefore a method to determine nicotine exposure needs to include measurement of metabolites of both nicotine and cotinine. This poster will demonstrate a simplified protocol for the simultaneous extraction of nicotine and its major metabolites prior to UPLC-MS/MS analysis. The analyte suite consisted of nicotine and its major metabolites: nornicotine, cotinine, norcotinine, 3-OH-cotinine and anabasine. Initial blow down results indicated nicotine volatility to be an issue with signal losses of up to 90%. This was rectified by addition of 50 mM HCl in MeOH to the collection plates prior to evaporation.

Supported liquid extraction method development involved investigation of optimum sample pre-treatment and extraction solvent combinations. Sample pre-treatment was evaluated using either 100 mM NH4OAc buffer (no pH), or 0.1-1% NH4OH concentrations. When using MTBE as the extraction solvent recoveries greater than 80% were observed for nicotine and anabasine while EtOAc also extracted cotinine to around 70% recovery. DCM demonstrated better recoveries for all analytes. However, a more polar extraction solvent combination of 95/5 DCM/IPA provides highest recovery of all analytes, greater than 70%. From a serum extract cleanliness standpoint the phospholipid content was investigated. Full results, discussion and conclusions will be shown in the final poster.

Keywords: Clinical/Toxicology, Liquid Chromatography/Mass Spectroscopy, Sample Preparation, Solid Phase Extr
Application Code: Clinical/Toxicology
Methodology Code: Sampling and Sample Preparation
Increasing Productivity by Utilizing Prepared Formulations

Formulations are prepared by various functions within a company. Departments performing this task include research and development, method development, quality control and the pilot plant. Companies are continuously looking for ways to apply lean manufacturing principles; typically used in the production area, to the other supporting functions within the organization. Depending upon the application, the process of preparing formulations in-house is often considered a non-value added activity. The task of making buffers and chemical solutions reduces productivity and creates hidden costs within a department.

This poster will examine the process of preparing formulations in the lab and identify the non-value added activities that are part of that process. These are the activities that decrease overall productivity and create hidden costs. Other factors that must also be considered when evaluating the preparation of formulations include worker safety, lot to lot formulation consistency, inventory management and reliability. 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 eliminate these wastes while addressing these other factors. When organizations choose to buy versus make their formulations, they are able to deploy their resources toward more value added activities, thus enhancing productivity. Honeywell LabReady Blends can be used from the R&D lab through quality control and into the manufacturing area. The poster will demonstrate how an organization that adopts Honeywell LabReady Blends can eliminate the non-value added steps required in formulation preparation, freeing resources for more value added activities.

Keywords: Chemical, Lab Management, Optimization, Sample Preparation

Application Code: Quality/QA/QC

Methodology Code: Sampling and Sample Preparation
Sampling and Sample Preparation

Advances in Tube Sampling Technology – Tube and Sample Data Tracking

Thermal desorption (TD) is a well accepted multi-stage sample preparation, refocusing and introduction technique for GC. Modern laboratory-based TD-systems automate most (if not all) of these stages, and can process large numbers of samples unattended. However, the logging, transfer and collation of large amounts of sample, tube and analytical data from field and laboratory observations is often spread between several systems, and it is usually a labour-intensive, error-prone task to relate data to individual samples.

This poster describes the design and implementation of a radio-frequency identification (RFID) tag system that allows information about the tube and sample to be kept with the tube throughout transport, sampling and storage. Lab-based TD systems equipped with RFID technology then allow the information to be transferred to the computer used for analysis, and modified accordingly, resulting in a file containing the full history of the sample.

Combining this technology with automated quantitative re-collection means that sample information can also be retained on re-collected samples. This allows – for the first time – both the sample and associated data to pass seamlessly from one tube to another. This is especially useful in cases where the same sample is run and re-collected successively under high- and low-split conditions to extract maximum information about the sample (‘High/Low analysis’).

Keywords: Sample & Data Management, Sampling, Scientific Data Management, Thermal Desorption
Application Code: High-Throughput Chemical Analysis
Methodology Code: Gas Chromatography/Mass Spectrometry
Static headspace sampling has always been a viable option for the detection of Volatile Organic Compounds (VOCs) and is readily used in Europe and Canada for testing water samples. In order to detect low level contamination of water, it is essential for the static headspace sampling and GCMS analysis parameters to be enhanced. This poster will examine automated headspace sampling of VOCs in water using a new innovative sampling system that performs sample preparation on top of the GC. The sampling and analysis will be optimized for better detection of volatile compounds at low concentrations and the final results will be compared with USEPA Method 8260 requirements.

**Keywords:** GC-MS, Headspace, Sample Handling/Automation, Volatile Organic Compounds

**Application Code:** Environmental

**Methodology Code:** Sampling and Sample Preparation
Polycyclic Aromatic Hydrocarbons (PAHs) are formed from incomplete burning of carbon containing fuel. There are thousands of PAH compounds in the environment and of those; there are several that have been established to be of concern for the environment. USEPA Method 3511 is a procedure for extracting selected semi volatile compounds from water that minimizes sample size and solvent use. This procedure in conjunction with the use of Large Volume Injection (LVI) allows laboratories the ability to inject larger volumes of sample without sacrificing sensitivity. This analysis will compare the linear range of PAH compounds using a standard injection versus a large volume injection.
Evaluating the Efficacy and Reproducibility of Automated Homogenization Technologies

Intro: Studies targeting small molecules typically start with a homogenization step. Past data suggests experimental variability and sensitivity is largely determined by differences in extraction efficiencies during homogenization. We evaluated the potential for automated homogenization to alleviate variation and improve reproducibility by comparing two automated homogenization systems to the common hand held rotor stator homogenization.

Methods: Two tissues were selected for the study: [i]Rattus norvegicus[/i] liver and brain. Tissues were sectioned into twenty four samples, eight for each platform, and diluted to 50 mg/mL in Tris-HCl, pH 7.6. Omni International’s Tissue Homogenizer (TH) fitted with a 7mm disposable Omni Tip generator probe was used to represent the hand held rotor stator method. Samples were homogenized on a medium setting in 14ml conical bottom tubes. Next, samples were homogenized with identical probes and tubes on the fully automated Omni International LH96 for 15secs at 30,000 rpm. Lastly, samples were homogenized on medium on the Omni Bead Ruptor 12 in 2ml tubes containing ceramic beads for 45secs. Post homogenization, samples were divided into two parts for protein and DNA extraction and quantification.

Results: Our study showed high yields for all platforms. The highly automated LH96 provided the smallest variance across sampling for both DNA and protein yields, followed by Omni’s TH, then the Bead Ruptor 24. SDS-PAGE analysis indicated a highly reproducible protein repertoire across all platforms. Past research on variance suggested homogenization accounts for more than 73% variation seen in LC-MS/MS proteomic analysis; however our data suggests this figure may be overstated. This novel study identifies for the first time, advancements in sample prep methods that provide efficient large, volume processing in automated manners.

Keywords: Automation, Biological Samples, Laboratory Automation, Sample Preparation
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Sampling and Sample Preparation
Abstract Text

Occupational Safety and Health (OSH) is an area concerned with protecting the safety, health and welfare of people engaged in work or employment. The goals of occupational safety and health programs include fostering a safe and healthy work environment. The National Institute for Occupational Safety and Health (NIOSH) is the U.S. Federal Agency for the prevention of Workplace Illnesses and Injuries.

The aim of this work is the screening of the volatile compounds according to the NIOSH 2549 method from NMAM (NIOSH Manual of Analytical Methods). This study was conducted using a system composed by two stage thermal desorber - gas chromatograph – time of flight mass spectrometer (TD- GC/TOF MS).

The analytical performance has been investigated. At first, a certain volume of sample is concentrated in a sorbent tube. This tube is heated and the volatile compounds are then desorbed by a flow of inert gas and transferred to a narrow-bore packed trap filled with one or more sorbent materials, and concentrated at low temperature. Once the refocusing step is completed, the trap is instantaneously heated providing a rapid analytes transfer directly into the GC/TOF MS system. Thermal desorption and gas chromatographic analysis is a suitable approach to perform an accurate and cost-effective monitoring. Data will be reported including chromatographic parameters for VOC and qualitative determinations.

Keywords: Clinical/Toxicology, Gas Chromatography/Mass Spectrometry, Sample Preparation, Thermal Desorption
Application Code: Industrial Hygiene
Methodology Code: Sampling and Sample Preparation
Five new polymeric stationary phases were developed for large sample volume solid phase extraction (SPE). The phase chemistries include a hydrophilic reversed phase material as well as mixed mode, strong and weak, anion and cation exchange. Base particles are grafted with polymeric and monomeric moieties to achieve a useful balance between reversed phase and ion exchange capacities. The hydrophilic reversed phase material bears specific functionality to retain polyphenolic and azo-containing species thru a charge transfer mechanism and H-bonding. This allows the retention of such species as humic acids along with the ability to elute other organic species using conventional reversed phase techniques. These new resins are formatted to be compatible with sample volumes larger than 20 mL in automated SPE instrumentation.

Several applications will be shown. The targets include triclosan, a popular but very hydrophobic bactericide, anionic and cationic surfactants, the weak base atenolol and others with specific advantage for the phases. Recoveries greater than 95% for sample volumes of 20-200 mL at loading flow rates of 5-30 mL/min in different matrices ranging from tap water, surface waters to the ASTM formulation of wastewater were observed. Comparisons illustrating the right choice of bed weight to minimize cost will also be presented.
Endothall is an herbicide that is commonly used in water to control algae growth. Because of its use in water, it is often present in drinking water and is regulated under EPA 548.1. In water samples that contain even low concentrations of other ions like calcium or sulfate, the recovery of endothall can drop to below 10% recovery. The method details dilution and treatment steps with EDTA to minimize the interference, but the extraction is still problematic and complicated by these extra steps.

This work details the use of an alternative extraction sorbent that achieves high recoveries, even under very high calcium and sulfate concentrations without the need for extra dilution or treatments steps. All other aspects of the method are unchanged so the alternative sorbent is not considered a deviation from the method. The improved procedure achieves greater consistency without the need to pre-screen samples for extra possible interferences.
Automation of a Solid Phase Extraction Method for the Determination of Ochratoxin A in Wine and Beer Samples Prior to LC-MS/MS

The emerging threats of mycotoxin contamination in our food, particularly ochratoxin A (OTA), have led to surveillance programs employing low-level screening methods in both industrial and government foods safety laboratories. The persistent nature of this contaminant has led to its determination in a range of processed foods including cereals, beer and wine. Of particular interest to this investigation is the utility of automation to facilitate large scale quality control efforts in the preparation of samples prior to analysis by liquid chromatography-mass spectrometry-mass spectrometry (LC-MS/MS). A solid phase extraction method using application specific polymer column technology called the ISOLUTE Myco (60 mg/3 mL column) was developed from a multivariate statistical design of experiment (DOE). This method was employed to determine OTA concentrations in Malbec wines from Argentina. The relative recovery for the extracted mycotoxin was 95-105% (<15% RSD) over a linear concentration range ($r^2 = 0.997$) of 0.4-4.0 µg/mL. The optimized method was automated using a Biotage RapidTrace workstation to ensure consistent method precision in high-throughput surveillance laboratories. Feasibility of the direct transfer of the offline chemistry to the automated system was evaluated.

**Keywords:** Laboratory Automation, Mass Spectrometry, Sample Preparation, Solid Phase Extraction

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

**Methodology Code:** Sampling and Sample Preparation
Laboratories that are involved in food analysis are often required to determine the percent fat that is present in their samples. These procedures often require use of a Soxhlet apparatus and nitrogen stream evaporation to accomplish a gravimetric determination of the percent fat content. These method are often laborious and require copious amounts of solvent per sample (>100 mL), extraction time per sample (> 1 hour), and access to a fume hood to accomplish evaporation. Recently, use of the Accelerated Solvent Extractor (ASE) with the Rocket Evaporator was investigated as an automated sample preparation workflow. The use of these two techniques together reduced extraction and evaporation time to less than one hour for a batch of six solvents and decreased solvent use to as low as 20 mL and extraction time to as low as 18 minutes per sample.

ASE uses elevated pressures and temperatures to effect rapid extractions with small solvent volumes. We will present the results of our work to extract fat from food matrices (e.g. chocolate and seeds) to develop an automated sample preparation procedure. Use of the ASE with the Rocket Evaporator automates both the extraction and evaporation to eliminate the need for cumbersome manual techniques. We will demonstrate the impact that automation generates for the workflow in fat determination from food matrices and how use of both products together substantially reduces the amount of solvent and time required to yield samples ready to gravimetric analysis.
Since its introduction in 2003, QuEChERS extraction has been adopted by many laboratories undertaking pesticide residue analyses on samples from fruit and vegetables to complex herbs and spices. Clean-up of extracts for the removal of matrix components that interfere with subsequent LC-MS/MS or GC-MS/MS analyses has mostly relied on dispersive solid-phase extraction (dSPE). However, for many matrices, co-extractants causing suppression or enhancement of analyte signals remain after dSPE, requiring the use of matrix-matched calibration standards, or spike additions, for accurate quantitation. Precipitation of oils can also occur, with addition of aqueous buffer for LC-MS/MS, taking non-polar analytes out of solution. Column SPE has been employed for more complex sample types, as it offers improved clean-up. This is labor-intensive and requires skilled technicians, therefore a novel robotic SPE clean-up was developed, using miniaturized cartridges. This can be placed on top of the instrument, and clean-up incorporated into the analytical run, saving time and labor. Experiments with a variety of sample types showed effective removal of interfering sugars, fatty acids, colors (chlorophyll and carotenoids), and oils for GC-MS/MS or LC-MS/MS analyses, with excellent recoveries of pesticide spikes presented. This has enabled use of solvent standards for calibration, and removed oil precipitation, allowing multiple sample types in one analytical run, with reduced instrument fouling, improving reproducibility over many injections.

**Keywords:** Isolation/Purification, Pesticides, Prep Chromatography, Tandem Mass Spec

**Application Code:** Agriculture

**Methodology Code:** Sampling and Sample Preparation
Gypsum board is a kind of drywall panel material made from gypsum plaster and is commonly used to make interior wall and ceiling in construction of house. In contrast, wallboard yields an estimated 14 million tons of waste annually and that waste would be increased. Scrapped gypsum drywall is currently being recycled as cement and fertilizer. However, some type of gypsum boards often contains arsenic compounds in the virgin materials. The monitoring or assay for arsenic compounds in the scrapped gypsum board has been demanded to prevent the contamination to environment. It is necessary to dissolve gypsum to quantify the impurity in gypsum boards. However, gypsum is mainly composed of calcium sulfate dihydrate which is difficult to dissolve in water directly due to its low solubility. Hydrochloric acid or perchloric acid has being commonly used for dissolving of gypsum. In this presentation, we presents a simple and quick dissolving method for gypsum boards using cation exchange resin. This method can dissolve gypsum in a little quantity of water within 5 min without any acidic chemicals. Cation exchange resins served to preferentially capture calcium ions and in contrast arsenic compounds as an analytical target were not slightly captured on the resin. In combination of this preparation method with simple digital arsenic test kit, the quantification of arsenic compounds in gypsum boards was achieved within 30 min. In addition, it was applied to the sample preparation for ICP-AES. The fact would be expected to the application ICP-MS or atomic absorption spectrometry.

Keywords: Environmental/Waste/Sludge, Ion Exchange, Method Development, Sample Preparation
Application Code: Environmental
Methodology Code: Sampling and Sample Preparation
Different materials like glass, silicon and PMMA are been used to immobilized enzymes into microchannels. PMMA shows some advantages such as its low price, biocompatibility and attractive mechanical and chemical properties. Despite that, introduction of reactive functional groups on PMMA is still a trouble either because of the complex chemistry or extended reaction time involved. In this context a simple, fast and cheap methodology was developed to immobilize glucose oxidase in PMMA microchannels. The procedure is based in the reaction of 5.0% (w/w) polyethileneimine (PEI) with PMMA into dimetilsulfoxide medium for 20 min. Glucose oxidase is then immobilized with a 200 U mL[^1] enzymatic and 1.0% (v/v) glutaraldehyde solution. Reactors efficiency was evaluated by a flow system with chronoamperometric detection (+0.60V) of H[^2]O[^2] produced by the reactor. A dual platinum electrode was employed as working and counter electrode and a miniaturized Ag/AgCl([sub]sat[/sub]) electrode was used as reference electrode. The flow system was composed by two pumps: a one way pneumatic propellant aquarium pump, used for the carrier electrolyte (0.10 mol L[^1] phosphate buffer, pH 7.00), and a programmable peristaltic pump, used to introduce reproducible volumes (10 [micro]L) into the microchannel. Statistical evaluations were done to validate the proposed methodology. Amount of glucose in coconut waters samples was evaluated using the developed method. Comparison with spectrophotometric measurements showed that both methodologies have a very good correlation (t[^calculated, 0.05, 4[/sub]] = 0.25 < t[^tabled, 0.05, 4[/sub]] = 2.13). Detection and quantification limits were 1.76 and 5.28 [micro]mol L[^1], respectively.

**Keywords:** Biosensors, Electrochemistry, Enzyme Assays, Lab-on-a-Chip/Microfluidics

**Application Code:** Bioanalytical

**Methodology Code:** Sensors
Microfluidics provide a versatile platform for chemical analysis, and as such have received much attention over the last 10 years with applications in biology, pharmaceuticals and materials science. Traditionally, microfluidic devices are fabricated from polydimethylsiloxane (PDMS) which although is advantageous for rapid fabrication, is limited to use with aqueous media due to incompatibility with non-polar, volatile solvents. Integration of electrochemical sensors into microfluidic devices is of growing interest with the advantages of reducing analyte material consumption with increasing detection sensitivities.

Synthetic diamond is unique as a microfluidic device material in that it is highly resistant to chemical attack, fouling and harsh environments. Devices made from diamond are extremely resilient and can withstand aggressive cleaning procedures. Also, it has been shown that conducting-diamond electrodes can be seamlessly integrated into intrinsic, all-diamond tubular flow devices. Here we describe the fabrication of an all-diamond microfluidic channel device with integrated Platinum band electrodes to enable electrochemical detection of fluid components.

References

Keywords: Electrochemistry, Lab-on-a-Chip/Microfluidics, Sensors, Voltammetry
Application Code: General Interest
Methodology Code: Microfluidics/Lab-on-a-Chip
Borate-based glasses are a new type of bioactive glass that has been developed for biomedical applications. Most recently, bioactive borate glass nanofibers have been fabricated for soft tissue applications. The nanofibers promote angiogenesis, making it a potential candidate for soft-tissue repair. The mechanisms of the wound healing function of the nanofibers are largely unknown. To study the mechanism, we have designed an in vitro dynamic flow control model system that mimics microenvironment of the vascular hyperplasia area in wound-healing region to demonstrate biological compatibility and functionality of the nanofibers. This developed dynamic flow control system was used to study how the bioactive borate nanofibers affect the cell growth, the major angiogenic growth factor (vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF)) secretion, borate release and speciation, and other elements released through the nanofibers and biological system interactions. Bioactive borate glass 1393B3 (53% B2O3, 20% CaO, 12% K2O, 6% Na2O, 5% MgO, 4% P2O5 (wt%)), with and without Cu/Zn modifications was used for this study. Silica based glass 45S5 (45% SiO2, 24.5% Na2O, 24.5% CaO, 6% P2O5 (wt%)) nanofiber was used for comparing. Cell growth was monitored by imaging system. The growth factors were detected by ELISA kits. Total boron and other elements were detected by ICP-OES and ICP-MS. The detail experimental design and results will be presented at the conference. This research was supported by Center for Biomedical Science and Engineering and Department of Chemistry in Missouri University of Science and Technology.

Keywords: Bioanalytical, Biomedical, Imaging
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Numerous compounds, including prostaglandins, uniquely mediate innate immunity and inflammatory responses throughout pregnancy to ensure proper gestation. One such vital mediator is prostaglandin E2 (PGE2), which governs the innate immune response to infectious agents and is found in high concentrations within the uterus. Studies suggest that PGE2 upregulation can influence the outcome of bacterial infections, however there is little known regarding the exploitation of the fetal membrane PGE2 response by bacteria to promote chorioamnionitis. The fetal membrane contains macrophages, which are known immunological regulators for the body. Previous work has shown that RAW 264.7 cells, a murine macrophage cell line, exhibit an increase in lactate production, oxygen consumption, and extracellular acidification (ECAR) shortly after exposure to the gram-negative effector, lipopolysaccharide (LPS). Interestingly, the synergistic effects of interferon-α (IFN-α) were immediately seen and resulted in a decrease in lactate production and ECAR while showing an increase in oxygen consumption, suggesting IFN-α primes the cell for efficient oxidative burst upon LPS exposure. Due to the quantitative effects of LPS on RAW cells, research was conducted to examine the response of THP-1 cells, a human-derived monocyte cell line that was differentiated into macrophages. The multianalyte microphysiometer (MAMP) was utilized to measure dynamic, simultaneous responses of glucose and oxygen consumption, lactate production, and ECAR of THP-1 cells undergoing exposure to LPS and PGE2/LPS. Additionally, a pre-exposure to PGE2 prohibits LPS from initiating oxidative burst in THP-1 cells, allowing for macrophages to sustain basal metabolism during a bacterial exposure. These studies provide real-time metabolic profiles that shed insight into fetal membrane resistance to bacterial infections, which could yield clinically relevant insight for future treatment.
### Abstract Text

Due to the complexity of eukaryotic system, cells display strong heterogeneous behaviors even under seemingly identical environmental conditions. Thus the obscure population-averaged experimental results have become questionable when fundamental inquiries about individual cell heterogeneity were raised up, such as cell deaths, differentiation and carcinogenesis, etc. Single cell level pH measurements were chosen for investigation for its crucial function in immediate response of a variety of cellular stimuli. A fibre-optic based pH sensor with fast/remote sensing capability was fabricated and tested. A single mode optical fiber (Corning SMF-28) taper with a center diameter ranged of 500 nm to 2 μm was fabricated using CO2 laser fiber-stretching system. A porous spherical shaped micro- or nano-scale taper tip was also fabricated using femto-second laser ablation. pH sensitive dye (BCECF) in free acid form were then covalently bonded through amino-group connectors onto TEOS-DIAMO-GLYMO based aerogel ultra-thin film which located on top of above-mentioned taper tip surface. A subsequent supercritical fluid drying process as well as a specific heat-treatment was employed for better pH sensing stability and resolution. Real-time sensing and imaging capabilities acquired through a NSOM system with high spectroscopical sensitivity. Ratiometric method was used in this study to quantify fluorescent peak shifting and intensity variations. A pH resolution of 0.1 was expected with sufficient reproducibility. As-fabricated fibre-optic pH sensor was then validated using a series of phosphate buffers with finely adjusted pH while its ultimate feasibility was tested with live human lung cancer cells (A549) and human fibroblast epithelial cells (CCL-110). More detail about the fabrication and intracellular measurement will be presented at the conference. This project was supported by national Institute of Health (Award #: R21GM104696).

### Keywords
- Biosensors
- Fiber Optics
- Fluorescence
- Laser

### Application Code
- Biomedical

### Methodology Code
- Integrated Sensor Systems
Tuberculosis is a dangerous disease and its death toll is increasing year by year. Intake of isoniazid and rifampicin lead to fatal hepatic and kidney toxicity. Male albino rats weighing 200 ± 20 g were treated orally for induction of hepatotoxicity with 200 mg/kg of INH and RIF daily each for 30 d. For hepatoprotective studies, 200 mg/kg/day of N-acetylcysteine (NAC), white tea (WT) starting from the 15th day were administered orally, while rat bone marrow mesenchymal stem cells (BMMSCs) was injected through the portal vein in a volume of 0.2mL containing 1×10⁶ cells at the 15th day. Results revealed that Serum liver enzymes activities AST and ALT, liver and kidney TRABS and NO levels, serum lipid profile and DNA damage which exhibited by COMMET assay were significantly increased by (INH+RIF) treatment associated with marked reduction of serum levels of albumin, protein and A/G ratio, as well as liver and kidney GSH levels and antioxidant enzymes activities as compared to normal controls. These results showed that (NAC), (BMMSCs) and (WT) extract exerts their protective activity by different extents on liver and kidney by inhibiting the production of free radicals through induction of antioxidant enzymes and improving non-enzymatic thiol antioxidant GSH and returning back the tested parameters towards near the normal controls. Results, also demonstrated that liver and kidney functions were improved greatly by (NAC) followed by (BMMSCs) and (WT) extract in the present study. Therefore, BMMSCs are a potential option for treatment of liver and kidney impairment.

Acknowledgements: We thank the NODCAR (National Organization of Drug Control and Research), Cairo, Egypt for financial support in the form of a Major Research Grant.

Keywords: Bioanalytical, Biological Samples, Clinical/Toxicology, Natural Products
Application Code: Biomedical
Methodology Code: Sampling and Sample Preparation
Cystic fibrosis, CF, is the most common genetic disease in the US. CF is caused by a defective chloride channel in the epithelia. Early screening for CF is a high-priority goal in public health. Various tests are being done in newborns for screening for the presence of cystic fibrosis but these tests are prone to major errors in diagnosis. The gold standard test is the measurement of chloride in sweat, however this test is difficult to implement in newborns due to the requirement of collecting on the order of 50-100 microliters of sweat. The sweat test is also labor intensive and difficult to do. Therefore there is a need for a new approach to chloride sweat test that requires very small volumes of sweat so that it can be done in newborns. It is also necessary for this test to be inexpensive if universal screening is the goal. We report on an approach that can perform the gold standard chloride test in 5 microliters of sweat or less which is easy to collect in newborns. The device consists of a tiny electrochemical sensing piece that is brought in contact with sweat directly on the skin. An attached small, portable device controls the electrochemical measurement using a chloride selective impedance based scheme, and reads the result. Data indicate that the linear range of sensitivity is broad enough to reliably test for the absence/presence (below 60 mM or above 80 mM, respectively) of cystic fibrosis.

Keywords: Biomedical, Biosensors, Electrochemistry
Application Code: Biomedical
Methodology Code: Portable Instruments
Photodynamic therapy (PDT) which requires the combination of a photosensitizer (PS), light and oxygen is a non-invasive therapeutic modality and widely used in cancer clinical trial. However, most existing PSs are hydrophobic in nature. The oxidative damage is therefore significantly reduced by low efficiency of reactive oxygen species (ROS) production. Owing to the high binding affinity of serum albumin toward PPIX, bovine serum albumin (BSA) was applied as a carrier for PDT drug in the current study. High aqueous solubility of BSA also makes it an ideal candidate to stabilize hydrophobic porous hollow Fe3O4 nanoparticles (PHNPs) through one step oil-in-water emulsion under optimal sonication condition. The morphology and particle size of the PPIX:BSA:PHNPs were characterized by transmission electron microscopy (TEM) as well as dynamic light scattering (DLS). The spherical nanocomposites demonstrated a narrow size distribution with a mean hydrodynamic diameter of 155 ± 7.2 nm. Fluorescence measurement also confirmed a high PPIX-loading efficiency and minimum drug leakage (< 1%) of the developed nanocarrier under physiological condition for 72 h. Furthermore, the in vitro cytotoxicity of PPIX:BSA:PHNPs was tested in HeLa cells by an MTT assay and a significant improvement in therapeutic efficacy could be achieved with 30 min-red laser (632 nm)irradiation. This result is consistent with an increase of ROS generation in cancer cells, as demonstrated using flow cytometry. Our findings suggest that albumin coated magnetic nanoparticles exhibit great potential as a drug delivery system for hydrophobic photodynamic sensitizers in cancer therapy.
Surface-enhanced Raman scattering (SERS) active optical fiber sensors combine the SERS substrate with optical waveguide, which allow the applications for in situ and long-distance SERS detections. The modification of SERS-active sensing layer is one of important topics in the development of new SERS active optical fiber sensors. In present study, we prepared a highly sensitive SERS-active optical fiber sensor by in situ polymerizing a porous polymer material on the optical fiber terminal end through a photo-induced process following with photochemical silver nanoparticles growth. The light sensitive polymer (poly (glycidyl metharylate-co-ethylene) dimethacrylate, GMA-co-EDMA) formed a three-dimensional pore structure on the surface of optical fiber, with a pore diameter of about 1 μm. The deposited silver by light reduction formed nanoparticles with a size of about 100 nm, which are monodisperse around the surface of polymer pores. This SERS-active optical fiber sensor has been applied for the determination of 4-mercaptopyridine (4-Mpy) and crystal violet. The lowest detection concentrations for 4-Mpy and crystal violet are both 1 × 10⁻⁷ M. The optical fiber sensor with sensitive SERS-reactive porous polymer is expected for online analysis and environment detection.

Funings: NSFC Grant Nos. 21073073 and 91027010, National Instrumentation Program (NIP) of the Ministry of Science and Technology of China No. 2011YQ03012408 and Open Project of State Key Laboratory of Supramolecular Structure and Materials(Grant Number SKLSSM201218).

References

Keywords: Biosensors, Method Development, Spectroscopy, Surface Enhanced Raman
Application Code: Bioanalytical
Methodology Code: Sensors
Isolated organ perfusion is a common laboratory technique for investigating pharmacology and basic physiology. It has recently seen a resurgence of interest from the organ transplantation community, a multi-billion dollar industry which requires organs to be delivered to recipients in the best possible condition to ensure good outcomes.

At present, each preservation device on the market uses their own bespoke systems for monitoring physiological flows through the organ under perfusion. Commercially available systems for monitoring the combination of temperature, pressure and flow rate required for smart feedback loops are often too costly, too bulky or are designed for non-biological ranges.

The SmartPipe system incorporates a resistance temperature detector, a platinum constant temperature anemometer and a piezoelectric bridge pressure sensor in a small through-flow package (2x2x3cm) to simultaneously monitor liquid temperature, pressure and mass flow at physiologically relevant ranges in both inflow and outflow channels. The sensors are coated with a physiologically inert polymer to prevent fouling and embolism formation when in contact with blood.

Future versions of the device will incorporate continuous oxygen and carbon dioxide sensors for to monitor the metabolic state of the organ under perfusion.
Graphene oxide (GO) is a monolayer material with sp2–bonded carbon atoms and some oxygen functionalities on its surface and edges. Owing to the structural characteristics of GO, it can be combined with a variety of proteins, enzymes and nucleic acids, thus it can be employed in various biomedical applications. We found that aptamer-conjugated gold nanoparticles (Apt-Au NPs) immobilized on GO (Apt-Au NPs/GO) can effectively inhibit the enzyme activity. In this study, GO was modified with 29-mer thrombin binding aptamer-conjugated Au NPs (TBA29-Au NPs/GO) by stacking. TBA29-Au NPs can effectively bind to thrombin and inhibition of the thrombin-mediated cleavage of fibrinogen to form fibrin by steric blocking effect. The inhibitory potency of the TBA29-Au NPs/GO was increased when it was combined with GO which possess high surface area and multivalent interaction between thrombin and aptamer molecules on GO. The TBA29-Au NPs/GO showed anticoagulant effect which is 5-fold higher than that of TBA29-Au NPs, and it can be used in control of the coagulation in blood. The easily prepared and low-cost TBA29-Au NPs has potential in biomedical applications for treating various diseases related to blood clotting disorders. In addition, this study opens up the possibility in graphene-based materials for regulation of molecule binding, protein recognition and enzyme activity by using aptamer-functionalized nanomaterials.
In recent years, there is increasing interest on electrical and electrochemical detection of nucleic acid for high-throughput and cost-effective DNA sequencing and genetic analysis systems. For quantitative analysis of small amounts of nucleic acid, the real-time PCR is widely used, in which thermal blocks for amplification, precision optics for detection and fluorescently labeled sequence-specific probes for target recognition are required. In this research, we propose to combine electrochemical detection using chronocoulometry (CC) with rolling circle amplification (RCA), which is one of isothermal amplification of nucleic acid, for simpler and more cost-effective genetic analysis. Chronocoulometry (CC) is one of the electrochemical techniques frequently used in electroanalytical chemistry for measurement of electrode surface area, diffusion coefficients, concentration, adsorption, and so on. We fabricated a patterned thin-film Au electrode for CC measurements. A self-assembled monolayer of alkanethiol was formed on the surface of the Au electrode with phi 29 DNA polymerase at constant temperature of 30 °C. RCA products was detected in situ using CC with [Ru (NH3)6]3+ as the signaling molecule which binds to anionic phosphate group along nucleic acid strand every three bases. We successfully detected CC signals for immobilization of ssDNA probes on the Au electrode, hybridization of ligated circle probe, and RCA products. We demonstrated that detection sensitivity for DNA and microRNA 143 were 500 fM and 50 pM, respectively. We further expect improvement of detection limit by optimizing probe design and assay conditions. Our results indicate possibility to provide in situ amplification and detection of nucleic acid for simpler and more cost-effective detection system for clinical research and diagnosis.
Human fungal infections have gained recent notoriety following contamination of pharmaceuticals in the compounding process, but such invasive infections are a more serious global problem, especially for immunocompromised patients. While superficial fungal infections are common and generally curable, invasive fungal infections are often life-threatening and much harder to diagnose and treat. The annual number of deaths from fungal infections is comparable to those from tuberculosis or malaria. Despite the increasing awareness of the situation's severity, currently available fungal diagnostic methods cannot always meet diagnostic needs. Volatile organic compounds produced by fungi provide an alternative diagnostic approach for identification of fungal strains. We show here an optoelectronic nose based on a disposable colorimetric sensor array capable of rapid differentiation and identification of pathogenic fungi based on their metabolomic profile of volatiles. The sensor arrays were tested with 12 human pathogenic fungal strains grown on standard agar medium. Array responses were monitored with an ordinary flat-bed scanner. All fungal strains gave unique composite responses within 3 hours and were correctly clustered using hierarchical cluster analysis (Figure). A standard jackknifed linear discriminant analysis gave a classification accuracy of 94% for 155 trials. Tensor discriminant analysis, which takes better advantage of the high dimensionality of the sensor array data, gave a classification accuracy of 98.1%. The sensor array is also able to observe metabolic changes in growth patterns upon the addition of fungicides, and this provides a facile screening tool for determining fungicide efficacy for various fungal strains in real time.

Keywords: Array Detectors, Bioanalytical, Biomedical, Chemometrics
Application Code: Biomedical
Methodology Code: Sensors
Sensors: Bioanalytical and Biomedical

Molecular Characterization of Extracellular Phytase-Producing Fungi by Using 18S rRNA Sequence Analysis

Phytate - phytic acid - is the main storage from of phosphorous in harvest (legumes, cereals and etc). Phytic acid decreases the absorption of metals in small intestine and affects bio-beneficialness. Phytic acid creates phytate-protein complexes in both low and high pH. These complex formations change the protein structure and also make proteins resistant against proteolytic digestion by blocking digestion enzymes. Recently, phytase have been of interest for biotechnological application.

In this study, we investigated the phytase activity of fungi from soil. Phytase producing fungi were isolated using phytase screen medium (PSM). Fungal isolates were selected according to their higher phytase activities. These isolates were further characterized and identified by morphological analyses and amplification of 18S rRNA gene, using specific primers.

Our study showed that isolated fungi strains have significant high activity of phytase. Therefore, this enzyme can be used in the animal feed industry. In future researchs, fungal genome can be used for overexpression of microbial phytase.

Keywords: Biotechnology, Genomics, Soil
Application Code: Genomics, Proteomics and Other 'Omics
Methodology Code: Sensors
Anthrax poses a significant threat to US National Security as demonstrated by the 2001 terrorist attacks targeting the US Postal Service and Hart Building. The causative agent, Bacillus anthracis, is ubiquitous worldwide. More importantly, it is found in countries harboring terrorists.

Anthrax outbreaks commonly occur in livestock. Consequently, the agent is routinely isolated, propagated, and maintained in laboratories by indigenous populations to diagnose the disease. This practice drastically increases laboratories’ repositories of B. anthracis, and escalates the risk that the agent can be stolen for nefarious purposes. Moreover, it enhances the capabilities of laboratory personnel to produce pure isolates.

To mitigate these risks, we have developed a robust portable diagnostic device for detection of bacteria in ultra-low resource environments that is low cost (<$5/assay), requires no power, instrumentation or equipment to operate, no cold chain to maintain efficacy, and can be operated by individuals with little to no technical training.

The self-contained credit-card sized device employs micro-culture methods to amplify bacteria prior to downstream lateral flow assay, improving detection limit by 4+ orders of magnitude (positive detection from 100 spore initial inoculum, Ames strain). Additionally, the device utilizes chemical and biological means to sterilize the contents following assay. Self-decontamination minimizes the potential for malicious use of the bacterial sample following assay and reduces the need for experienced staff to isolate the organism in the laboratory.

Finally, this platform is readily adaptable for detection of other bacterial agents including Burholderia pseudomallei, food borne pathogens, and drug resistant Staphylococcal and Streptococcal organisms.

Keywords: Biosensors, Environmental/Biological Samples, Immunoassay, Lab-on-a-Chip/Microfluidics
Application Code: Homeland Security/Forensics
Methodology Code: Bioanalytical
Short strands of RNA, called microRNAs (miRNA), participate in post-transcriptional gene regulation and have been identified as biomarkers of disease. Detection of these miRNA with biosensors is complicated by nuclease degradation of the biosensor causing false positive signals. We have developed an innovative biosensor to address this problem. The biosensor consists of two partially complementary strands of DNA that form a complex. One strand, called the reporter, contains two dyes capable of energy transfer located on opposite ends of the strand. The other strand, called the probe, is partially complementary to the reporter and is fully complementary to the target miRNA. When the target miRNA is presented to the reporter-probe complex the reporter is displaced and a probe-target complex is formed. The displaced reporter forms a hairpin configuration to bring the dyes together. This causes a quantifiable change in analytical signal dependent on miRNA concentration. We will show the reporter-probe complex selectively detects target miRNA in cell lysate at room temperature. Use of carbon chain spacers between the dyes and the nucleic acids have been investigated to optimize energy transfer efficiency when the reporter is in the hairpin structure. We are currently exploring cellular delivery techniques for cellular analysis of biomarker expression.

Keywords: Biosensors, Fluorescence, Nucleic Acids, Spectroscopy
Application Code: Bioanalytical
Methodology Code: Sensors
Cancer has been a major burden of human health. Targeted drug delivery has improved cancer therapy. However, Multiple Drug Resistance (MDR) remains a serious obstacle for efficient drug delivery into target subcellular organelles, such as nucleus. Drug nanocarriers have shown potential to overcome MDR in targeted cancer therapy by protection of drugs from being pumping out or enzymatic inactivation. Previously, we have developed monodispersed DNA hydrogel nanoflowers (NFs) via noncanonical self-assembly driven by liquid crystallization of DNA generated from Rolling Cycle Replication (RCR). In the present study, we report 1) the integration of cancer-targeting aptamers into NFs, and 2) a novel platform of aptamer-integrated DNA NFs for inhibition of MDR in targeted anticancer drug delivery. By tailor-design of RCR template, aptamer KK1B10, which can specifically bind to MDR leukemia cells, was integrated into the resultant KK-NFs. KK-NFs were size-tunable, and KK-NFs with diameters of about 250 nm were assembled within 3 hours and used in subsequent study. Flow cytometric analysis showed that KK-NFs retained the ability to specifically bind to target cells, but not to nontarget cells. The hierarchical structures of KK-NFs endow them to both encapsulate drugs and protect the encapsulated drugs from being pumping out or enzymatic inactivation. A widely-used anticancer drug, Doxorubicin (Dox), was laden into KK-NFs to be delivered into target MDR cancer cells. For optimal therapeutic efficacy, Dox needs to enter nucleus. And confocal microscopy study has demonstrated enhanced Dox retention in nucleus of MDR cells treated with Dox-laden KK-NFs, compared to free Dox. A cell proliferation MTS assay directly proved that the inhibition of MDR cancer cell proliferation was significantly enhanced by Dox delivered via KK-NFs, compared to free Dox. Furthermore, KK-NFs did not induce much cytotoxicity in nontarget cells. Collectively, KK-NFs are promising for MDR cancer therapy.

Keywords: Biomedical, Drugs, Nanotechnology, Nucleic Acids
Application Code: Biomedical
Methodology Code: Materials Science
Sensors: Bioanalytical and Biomedical

Label-Free Real-Time Chemical Observation of Living Cells Using a New CCD-type Ion Image Sensor

We have developed two-dimensional array CCD-type ion image sensors using complementary metal-oxide semiconductor (CMOS) and charge coupled device (CCD) technologies. Recently, we successfully developed a new CCD-type sensor with 128 x 128 (16384) sensing pixels in about 5 mm square, and the ion images were obtained every 0.03 s (Fig.1). The ion image sensor has attractive features; Quantitative image of ions, Compact equipment (227x168x160 mm), Easy handling, Without probes (label free), Fast response (33 fps), Able to record data and to create a movie, Wholly fabricated by CMOS technology. The Si$_3$N$_4$ membrane of the basic substrate responds to pH, and the membranes substituted by several plasticized poly(vinyl chloride) respond to biologically important ions.

In the presentation, we demonstrate our recent two results for bio-imaging by the new CCD-type ion image sensor. One label-free real-time observation is histamine release from rat mast cells stimulated. The plasticized PVC membrane responded to biogenic amines such as histamine and serotonin. The other is potassium ion release from a slice tissue cultured of rat hippocampus by a stimulus. The potassium ion sensitive plasticized PVC membrane was utilized. These images obtained can provide a new sight to the bio-imaging world.

Keywords: Bioanalytical, Imaging, Ion Selective Electrodes, Sensors
Application Code: Bioanalytical
Methodology Code: Sensors
Adenine is one of the most important organic molecules for life. It is an integral part of DNA, RNA, and ATP. Adenine quantitation is required for a variety of applications. To date, adenine is measured either indirectly by detection derivatized adenine, such as ATP, or phosphate (after a series coupling enzymes: adenine phosphoribosyl transferase (APRT), pyruvate orthophosphate dikinase (PPDK), 5’-nucleodidase converted from adenine) as its end point, or directly by HPLC. However, these methods are either time consuming or complicated. For the present study, we developed a high-through put (HTS), nonradioactive, enzyme spectrophotometric assay using xanthine oxidase as a coupling enzyme. This assay does not require adenine derivatization and is designed for microplates. Adenine is oxidized by xanthine oxidase, producing 2, 8-dihydroxyadenine. The molar extinction curve of adenine has an absorption maximum at 260nm, whereas the absorption maxima of 2, 8-dihydroxyadenine lies at 305nm. Because other compounds usually present in biological filtrates are low at this wavelength (305 nm), and because of the sensitivity and the optical density of most proteins, adenine can be detected by monitoring the spectrum absorption change at 305nm. The measured sensitivity of adenine and its substrate specificity is comparable to that determined by the HPLC detection method.
Current methods for planning therapy for many cancer patients use combinations of data like tumor grade, stage, lymph node status, and presence of receptors for hormones and apply therapy guidelines applicable to the relevant tumors. Therapy based on the classification of a patient’s cancer permits selection of treatment regimens that provide the best outcomes for groups of patients with similar tumors. Unfortunately, the therapies selected may not be effective for all patients in these groups. It is likely that subtle differences between individual cancers, even those of the same classification, makes each cancer unique and causes variations in individual responses to therapy. Because most therapy agents induce direct or indirect damages on DNAs, quantifying the number of DNA damage that is generated in the circulating tumor cells (CTCs) will help in predicting the sensitivity of a patient’s cancer to certain chemotherapeutic agents. In this work, we are developing an automated integrated fluidic bio-processor that can isolate various CTC sub-populations from a patient’s peripheral blood, immunophenotype these cells, collect the DNA and quantify the number of DNA lesions as a measure of patient’s response to therapy. A major component of our device consist of a mixed-scale ([micro]m to nm) fused silica based fluidic system. DNA is channeled through the device possessing an input funnel populated with pillars for pre-stretching, a nanochannel to stretch the input DNA to near its full contour length and two-pairs of nano-electrodes (5 – 50 nm size) positioned orthogonal to the long axis of the nanochannel to directly read the length of the DNA for the frequency of DNA lesions and fragment lengths with exquisite resolution. With this information, it will be possible to predict the patient’s response to therapy and what drug regimen can be prescribed.
Localized surface plasmon resonance (LSPR) has emerged as one of the most sensitive, label-free biosensing techniques. Traditional LSPR-based biosensing utilizes the sensitivity of the plasmon frequency to changes in local index of refraction at the nanoparticle surface.1 Alternatively, biosensing devices that make use of plasmonic coupling hold promise of greater sensitivity, specificity and resistance to biofouling.2 Here, we introduce the idea of carrying out biosensing with protein-nanoparticle arrays, in which the conformation of the protein mediates plasmonic coupling of the nanoparticles attached. Initial studies were carried out by synthesizing small (10-20nm) citrate capped gold nanoparticles3 and subsequently binding them to collagen I fibrils. Addition of analytes such as acid, salt and sugar induce changes in the collagen structure, promoting changes in plasmonic coupling by increasing or decreasing the space between nanoparticles. These plasmonic changes can be detected visually and through UV-vis.


Keywords: Biosensors, Nanotechnology, UV-VIS Absorbance/Luminescence
Application Code: Bioanalytical
Methodology Code: UV/VIS
DNA molecules, known as important materials for genomic and structural purposes, are also useful in other applications such as the development of new diagnostics and materials. Boronic acid is well-known by its strong interactions with diols, alcohols, and nucleophiles. Therefore incorporation of the boronic acid moiety into DNA could lead the discovery of new scaffolds for diagnostic and therapeutic applications. Herein, we describe a series of our work in synthesis and incorporation of modified nucleotide (thymidine) with a “click handle” for incorporation of boronic acid into DNA.
DNA aptamers are DNA oligonucleotides that specifically bind target molecules, with a great potential of application in medical and research area. Aptamers are generated via a technique named Systematic Evolution of Ligands by Exponential enrichment (SELEX). Despite multiple advantages DNA aptamer has beyond antibody, too few paring modes and the resulting lack of diversity in natural DNA make it limited in information content and sometimes hard to create an aptamer. Considerable effort has been put towards increasing the information content of the DNA library by adding new type of nucleotide. An Artificially Expanded Genetic Information System (AEGIS) is a class of DNA that contains a number of artificial nucleotides by rearranging hydrogen bond donor and acceptor groups joining pyrimidine and purine analogs as the nucleobases to allow them to form pairs having the same geometry as A:T and G:C pairs, but with the different hydrogen-bond patterns. Owning to some innovations developed for this system, including DNA synthesis, PCR amplification and sequencing technologies, these new nucleotides now can be readily exploited in Cell-SELEX technique and incorporated into DNA aptamers. In this project we are seeking to incorporate these AEGIS nucleotides into DNA library and conduct the SELEX experiment, with the purpose of finding an aptamer against liver cancer cell. Afterwards the aptamer gained will be characterized to determine the affinity and specificity. A Se-derivatization of selected aptamer will be crystalized, to help understand the role of AEGIS nucleotides in aptamer structure.

Keywords: Bioanalytical, Biomedical, Biotechnology
Application Code: Biomedical
Methodology Code: Bioanalytical
Sensors: Bioanalytical and Biomedical

Abstract Title: Single Molecule Enzyme-Linked Immunosorbent Assay for Cytokine Detection with Ultrasensitivity

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Abstract Text:
Cytokines, the proteins with low molecular weight, are secreted by the immunologic system. It is reported that the serum concentration of cytokines in body fluids indicates on physiological or pathological state of the patient and can be used for prognosis and disease diagnosis or for the monitoring of treatment response, and meanwhile the determination of a broad set of cytokines can serve as a fingerprint for a particular disease. However, in body fluids of healthy individuals and at early stage of a disease many cytokines are present in the concentration that is below the limit of detection of current methods. Here we present a successfully developed digital ELISA-Single Molecule Arrays (SimoA) assay for cytokines detection. By loading the bead-based sandwich-like single enzyme-linked immunocomplex into 46-fL reaction wells on a 50,000 well optical fiber bundle platform, we are able to detect the target proteins at subfemtomolar concentrations, i.e., 200- to 1000-fold more sensitive than conventional ELISA technology. Currently, 8 cytokines including GM-CSF, TNF-alpha, IFN-gamma, IL-2, IL-4, IL-1beta, IL-6 and IL-10 are demonstrated detectable in health human serum samples, most of which were previously undetectable using existing approaches. It is believed that our technology will be a milestone in the human disease diagnostic field.

Keywords: Bioanalytical, Biomedical, Biosensors, Clinical Chemistry
Application Code: Biomedical
Methodology Code: Biomedical
The concept of capacitive micromachined ultrasound transducers (CMUTs) came as an alternative for conventional ultrasound transducers based on the piezoelectric effect [1]. The use of CMUTs in sensing applications involves mass resonance sensing approach, which gives extreme sensitivity in the gaseous medium due to low moving mass and good resonance quality of the CMUT structure. The CMUTs demonstrated attogram level sensitivity per single element and such a high sensitivity is very useful for the design of immunosensors and other affinity-interaction-based sensors [2,3].

In the current work we aim to analyze the relationships of the CMUT surface modification methods and measurement conditions with the changes of CMUT resonance frequency and resistance, which were identified earlier as potentially informative parameters that can give adequate sensing performance. In our study cMUT micromembrane arrays modified with adsorbed bovine leukemia virus antigen were applied as a biological recognition part and the detection of specific antibody conjugate with horse radish peroxidase was performed. For the confirmation of the formed immune complexes on the surface, tetramethylbenzydine was added as an enzyme substrate. The results presented here illustrate that CMUTs are potentially suitable for immunosensor design.

Acknowledgements
This research was funded by a Grant No. MIP-059/2012 from the Research Council of Lithuania.

References

Keywords: Bioanalytical, Immunoassay, Sensors
Application Code: Bioanalytical
Methodology Code: Sensors
Multiplexing is a straightforward and effective method to collect large sets of biological data with high throughput and reduced assay-to-assay variation. Various detection probes have been developed in signal output, such as fluorescent tags, mass tags, radioactive tags, etc. In this presentation we describe the use of diRuthenium organometallic complexes as electrochemical tags for multiplexed bioassays (Scheme 1) for their good sensitivity and versatility. The advantages of using diRuthenium complexes as electrochemical tags are two folds: (a) the resulting redox potentials of diRuthenium complexes are tunable by synthetically modifying the complex ligands and, more importantly, (b) some complexes exhibit multiplex redox potentials that can be used collectively to reduce detection false positives from artifacts.

The concept of electrochemical multiassays is first demonstrated using Ferrocene derivatives for their ease of handling. Successful detection of multiple DNA targets is achieved. We then progress to demonstrate the use of Ru2(ap)4(OPE) (Compound 1) which is pre-derivitized with a TMSE (trimethylsilylethyl)-protected thiophenyl at the distal end for DNA attachment. The presentation will focus on the experimental optimization of compound 1 attachment chemistry and electrochemical characterization of the probe in the absence/presence of DNA molecules. Careful experimental controls over water and oxygen contents in the reaction mixture are examined for reproducible reduction with TBAF (tetrabutylammonium fluoride). The attachment to amino-DNA probes is realized with SMCC coupling reaction with high efficiency. Stable cyclic voltammetric scans are carried out for the immobilized species in 0.1M KCl solution in water, though diRu complexes themselves are not water soluble. A sandwich DNA complex is formed after target hybridization, bringing Ru2(ap)4(OPE) close to the working gold electrode surface. The lengths of DNA spacers and surface blocking reagents are optimized to balance electron transfer efficiency and steric hindrance to hybridization. We also present here an investigation of the effect of diruthenium compounds on the hybridization specificity and kinetics. Scheme 1: Schematic illustration of a DNA duplex formed on a Au electrode surface with a diRu probe attached at the distal end for detection. purple DNA strand: capturing strand; green DNA: targeted strand; brown DNA: probe strand.

Keywords: Biosensors, Chemically Modified Electrodes, Electrochemistry, Electrode Surfaces
Application Code: Biomedical
Methodology Code: Sensors
Determination of glucose in body fluids is an important analytical challenge in diagnostic analysis, especially in the case of continuous monitoring of glucose level in diabetes mellitus patients (1). Although enzymatic assays have been generally used for glucose determination, the most serious problem is its lack of enzyme stability, due to the intrinsic nature of the enzyme, the activity can be easily affected by various factors such as temperature, pH, etc (2). Because of this reason, new molecular recognition systems for the detection of glucose molecule have been attracting considerable attention (3).

A molecular recognition agent, phenylboronic acid, can recognise cis–diol configuration in saccharides and form reversible covalent complexes with saccharides in aqueous media, representing an ideal synthetic molecular receptor (4).

In this study, 3-aminophenylboronic acid was polymerized with benzoyl peroxide in 1,4-dioxane solution by chemical polymerization method. Obtained polymer was characterized by FT-IR, 1H-NMR, UV-Vis. spectrometry, elemental analysis and SEM techniques. The number average molecular weight, Mn, of the polymer was determined by gel permeation chromatography (GPC) and it was found to be 34200 g/mol. Poly(3-aminophenylboronic acid) was used to construct the organic film electrode for the determination of glucose by potentiometric and SERS measurement.

1. G. Reach, G.S. Wilson, Anal. Chem. 64 (1992) 381A–386A.

Keywords: Chemically Modified Electrodes, Electrode Surfaces, Polymers & Plastics, Potentiometry
Application Code: Polymers and Plastics
Methodology Code: Sensors
Avian influenza has caused enormous economic losses and posed continuing global human public health risks. Currently available methods for detection of avian influenza virus mainly includes virus culture and RT-PCR, but they are either time consuming or require specialized laboratory facilities and highly trained technicians. Simple, rapid, robust, and reliable tests, suitable for use in the field or at the patient’s bedside, are urgently needed. The purpose of this study is, based on our previous research, to develop a lower-cost impedance biosensing system with a redesigned microfluidic biochip and evaluate the improved biosensing system with inactivated H5N1 virus and chicken’s tracheal and cloacal swab specimens by comparing it with real-time reverse transcriptase PCR (rRT-PCR) and virus culture. This biosensing system consisted of a microfluidic biochip with an anti-H5 antibody modified interdigitated array microelectrode and a sub-millimeter-scale flowcell for transferring the aqueous samples and capturing the target viruses, an improved impedance detector with a wider frequency range for measuring the impedance, and a microfluidic control system with a peristaltic pump for accurately pumping solutions into the biochip. A comparison of results obtained from swab specimens using virus culture, rRT-PCR and impedance biosensing methods showed that this impedance biosensing system was comparable in sensitivity and specificity to rRT-PCR for detection of avian influenza H5N1. The whole detection time for a swab sample test is less than 2 h and the cost for a test is reduced by more than 75%.
Crimes related drug abuse and drug trafficking are serious global problems. Therefore, it is important to detect illegal drugs on-site. The most common method of drug-testing is gas chromatography coupled with mass spectrometry (GC/MS). However, rapid and simple screening methods are highly requested by customers and Border Protection. We found a novel fluorescent biosensor based on the antigen-dependent removal of a quenching effect on a fluorophore attached to antibody domains. This phenomenon was attributed to the interaction between a fluorophore and conserved Trp residues in antibody variable regions. Based on this observation, we named a pin-point fluorolabeled antibody as “Quenchbody” or “Q-body”. Our Q-body assay only requires us to mix Q-body with the sample and a reaction will be completed in a few seconds. Thus, Q-body is obviously an ideal biosensor protein for the on-site testing.

Here, we attempted to synthesize Q-bodies from sequence information of monoclonal antibodies specific to illegal drugs such as morphine, methamphetamine, and cocaine. These plasmids each encoding Fd or L chain gene for anti-drug antibodies were constructed, and two Q-bodies containing TAMRA in the N-terminal region of the H chain and/or L chain were synthesized in a cell-free translation system. The purified TAMRA labeled Q-bodies were mixed with the drugs, and fluorescence intensity increased immediately after mixing of them. All Q-bodies were tested for the binding activity against illegal drugs. As a result, Q-body shows a significant fluorescent enhancement as an amount of antigen increases in a few seconds.

Furthermore, we created a handheld on-site testing device for Q-body assay and sampling tool for drugs as Q-body assay system, and succeeded in a rapid and simple detection on-site for drug powder and liquid. Due to its versatility, this "Q-body assay system" can be used by Customs and Border Protection for a rapid and simple screening and on-site arrests by them.

Keywords: Biosensors, Fluorescence, Immunoassay, Integrated Sensor Systems
Application Code: Bioanalytical
Methodology Code: Portable Instruments
Characterization of Lauryl Acrylate Porous Polymer Monoliths used as Stationary Phases in Capillary Electrochromatography

Organic porous polymer monoliths (PPM) represent a new type of stationary phase, different in performance and structure from silica based monoliths and traditional particulate based phases. Used in a capillary electrochromatography system, these materials behave in interesting ways and are capable of producing quality separations. PPM materials have only been partially characterized. By investigating a lauryl acrylate PPM, we hope to better understand its behavior, and by comparison, the behavior of traditional phases. Studies have involved the examination of the relationship between retention and diffusion of analytes on these PPM phases, as well as the thermodynamics of retention. The diffusion study utilizes the peak-parking method during which the analytes are allowed to “sit” inside the column for a certain time period. The Knox equation, (Knox and Scott, J. Chromatogr. 282, 1983, 297), D_{eff} = (\gamma_m D_m + k\gamma_s D_s)/(1 + k) is one approach for examining the retention - diffusion relationship. The PPM columns used in this study follow Knox behavior, unlike traditional columns. The thermodynamic study indicates that retention is driven by enthalpy rather than entropy considerations. There is a less dramatic change in enthalpy than is seen with some traditional phases. This work is supported by the National Science Foundation, the American Chemical Society Project SEED and Petroleum Research Fund, the Welch Foundation, and the Howard Hughes Medical Institute.

Keywords: Capillary Electrophoresis, Characterization, HPLC, Separation Sciences
Application Code: General Interest
Methodology Code: Separation Sciences
Techniques for Reducing the Effects of Sample Solvents on UHPLC Analyses

For HPLC, sample solvents are required to dissolve target compounds satisfactorily. Therefore, sample solvents which contain a high concentration of organic solvents are often used for reversed phase chromatography. The problem is that those solvents sometimes cause peak broadening. In this poster, we discuss techniques for reducing the effects of sample solvents. Recent widespread UHPLC analyses are taken as examples. Reducing injection volume is a simple technique. To examine the effects of sample solvents, we injected 0.5-3μL volumes of samples on a UHPLC analysis with a methanol sample solvent and a mobile phase of water/methanol=70/30. Peak broadening was observed even when injecting only 2μL of sample. In addition, an increase in sensitivity was not observed when the injection volume was increased from 1μL to 3μL due to peak broadening. We also evaluated the reproducibility of peak area on some autosamplers. When injecting small volume such as less than 1μL, one autosampler could not achieve good reproducibility. On the other hand, the SIL-30AC autosampler, which is the autosampler of Nexera series from Shimadzu, showed reproducibility of less than 1% even when injecting only 0.1μL of sample.

Diluting samples with a weak solvent is also a good chromatographic technique. There is a problem in that diluting processes are tedious and time-consuming, but the SIL-30AC is equipped with a pretreatment and an over-lapping injection function. The pretreatment function enables automated dilution and the over-lapping function makes it possible to start the pretreatment operations for the next analysis while the current analysis is in progress. In the UHPLC analysis above, we diluted the sample ten times with water and were able to inject 50μL of the diluted sample without peak broadening, which resulted in a five-time increase in sensitivity.

Keywords: Automation, HPLC, Liquid Chromatography
Application Code: Other (Specify)
Methodology Code: Liquid Chromatography
Glycoproteins of biological, diagnostic or therapeutic interest owe key aspects of their normal function to the oligosaccharides attached to the protein backbone. 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 HILIC separation mechanisms. The native glycans were detected directly by using charged aerosol detection without derivatization.

Glycans released from various proteins were analyzed including those from bovine fetuin and monoclonal antibodies. Precision, detection limits and dynamic range of quantitative measurements are presented. The method was also used to study the completeness and side reactions of common fluorescent labeling reactions. Typical figures of merit include low-nanogram on-column sensitivity, four orders of magnitude of dynamic range, 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 both native and fluorescently labeled glycans, yielding simple, accurate and precise estimates of relative concentration even in the absence of pure primary standards.

**Keywords:** Biopharmaceutical, Carbohydrates, Characterization, Detection

**Application Code:** Pharmaceutical

**Methodology Code:** Liquid Chromatography
### Abstract Text

Hydrophilic Interaction liquid Chromatography (HILIC) proposed by Alpert in 1990 has been applied for analysis of hydrophilic compounds. Amide, diol, polyol, bare silica, ion exchange and zwitter ion phases have been used as a hydrophilic stationary phase along with an organic solvent rich mobile phase. A polar group embedded C18 or a long alkyl chain phase such as C30 or C28 also have been used to separate hydrophilic compounds with an aqueous mobile phase on a reversed-phase mode. The reason why these reversed-phases can be used under an aqueous condition is that a low contact angle of water on the surface of the pore of these reversed-phase packing materials makes an aqueous mobile phase keep in the pore, so that retention doesn’t change. Both HILIC stationary phases and reversed-phases have completely opposite characteristics each other. Therefore both HILIC and reversed-phase modes are useful for separation of hydrophilic compounds. It is important to understand separation behavior of each mode. In this study, an amide column and a C28 column were compared and evaluated to separate hydrophilic compounds. SunShell HILIC-Amide and Sunniest RP-AQUA (C28) and SunShell RP-AQUA (C28) were used to separate nucleobases, amino acids and hydrophilic vitamins. When nucleobases were separated on HILIC and reversed-phase modes using an amide column and a C28 column, each elution order of samples is said to be opposite. Only uracil, however, showed a specific elution. It was considered that the polarity of uracil under an organic solvent rich condition was different from that on water rich condition to be due to keto-enol tautomerization. LC/MS analysis of amino acids was achieved using C28 column and a mobile phase added 5 mM heptafluorobutyric acid under gradient elution conditions.

### Keywords
- HPLC Columns
- General Interest
- Liquid Chromatography
Hypertension is a medical condition characterized by high blood pressure. It leads to heart attacks, strokes, arterial disease and therefore reduces one's longevity and quality of life. There is a great interest in drugs used to manage hypertension. Hydrochlorothiazide is a drug that belongs to the class of diuretics used to treat hypertension and edema by inhibiting the ability of kidneys to retain water. A simple, accurate and precise reversed-phase liquid chromatography method was developed and validated to analyze Hydrochlorothiazide in the raw material and determine impurities and degradants. A method was developed on a 15 mm Luna Column C8, Particle size: 3µ, I.D. 4 mm under ambient temperature. Mobile phase used consisted of Monobasic Potassium Phosphate at pH 2.9 and Acetonitrile at a flow rate of 1.0 ml/min with UV-Vis detection at 273 nm. The separation was conducted under isocratic elution with 7/93% ACN/Buffer for 20 min. Hydrochlorothiazide was subjected to acid hydrolysis, base hydrolysis, hydrogen peroxide oxidation, heat and UV light. The Hydrochlorothiazide active ingredient was separated from the potential degradants, which were separated from each other. The developed method was validated for specificity, robustness, linearity, accuracy, precision, limit of detection and limit of quantitation. The method proved to be simple, accurate and precise with 98.7 % recovery of the active ingredient when tested on tablets of Lisinopril and Hydrochlorothiazide drug product.

Keywords: HPLC, Method Development, Pharmaceutical, Validation
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Interest in chromatography using hydrophilic interaction liquid chromatography (HILIC) has continued to build in recent years. 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 HILIC has been demonstrated. The aim of this continuing study was to further enhance our knowledge of mechanisms of retention through fundamental investigations of factors contributing to robustness and repeatability.

Although HILIC chromatography is known to provide valuable retention and selectivity of polar compounds and provide highly compatible conditions for coupling with mass spectrometry, it often is avoided due to issues surrounding robustness and repeatability. In this study, systematic investigations of possible contributions to repeatability are reported. Among others, the impact of sample solvent and equilibration procedures on retention times are investigated using several HILIC stationary phases. The results not only promise to improve method development practices, but also provide valuable insight into HILIC retention mechanisms across a diverse set of polar stationary phases.

Keywords: HPLC Columns
Application Code: Other (Specify)
Methodology Code: Liquid Chromatography
An important contribution to the high efficiency of core-type silica particles arises from a very low eddy diffusion or multipath term in van Deemter plots. A very narrow particle size distribution (PSD) of 6% RSD has been achieved for 2.7µm Fused-Core® silica particles, which may simply create a more consistent column bed and uniform sample flow path than particles having broader distribution. If small, porous silica particles having a similar, narrow PSD can be made conveniently and economically, they may allow routine preparation of columns with performance advantages much like core-type silica particles (1). Porous silica manufacturing usually delivers a broad particle size range that requires sizing before efficient, stable HPLC or UHPLC columns can be prepared. A new process has been developed to create silica particles that are already monodisperse and ready for use in HPLC or UHPLC columns, thus eliminating a wasteful and costly sizing process. The unique process allows preparation of TitanTM columns with monodisperse silica particles in a range of different diameters and pore-sizes.

Specifications, performance and applications for a UHPLC column with monodisperse, porous, 1.9 µm C18 silica will be described. Performance of TitanTM columns with new monodisperse silica will be compared to C18 columns with broader PSD in the same size range. Reduced plate heights below 2 and efficiencies of ca. 300,000 plates per meter will be shown for Titan C18 1.9µm columns. With Titan columns, the performance of UHPLC instruments can be improved while keeping pressure significantly lower than other sub-2µm columns.

Keywords: HPLC, HPLC Columns, Liquid Chromatography, Pharmaceutical
**Abstract Title**: Ultrafast 2D-RPLC: Superficially-Porous 2.7 µm Particles versus 1.8 µm Fully Porous Particles for Use in Second Dimension

**Abstract Text**
In ultrafast online comprehensive two-dimensional liquid chromatography (LCxLC), the second dimension must be much faster than the first dimension to ameliorate the loss in peak capacity due to undersampling. The goal of this work is to compare the separation performance of fully and superficially porous particles columns under conditions that are similar to those used for the second dimension in fast online LCxLC i.e. ultrafast separation at high temperature and under very steep (fast) gradient conditions and very high linear velocities. Experimental and theoretical calculated (based on isocratic experiments) peak capacity values are used to compare the performance of small columns (2.1 mm × 33 mm) packed with 2.7 µm superficially porous particles and 1.8 µm fully porous particles in very fast gradients (9 s to 2 min) and high temperature (95°C) at the same flow rate (1.75 mL/min) and at the same pressure (400 bar).

**Keywords**: High Temperature, HPLC, HPLC Columns, Liquid Chromatography

**Application Code**: Other (Specify)

**Methodology Code**: Liquid Chromatography
HILIC and Mixed-Mode are terms originally used to describe a specific chemical interaction or set of interactions in liquid chromatography. Recently, however, these terms have been more frequently used to describe LC columns offering retention mechanisms that vary, or are orthogonal, to a C18. These columns generally consist of a bonded phase which contains a chemical moiety other than, or in addition to, alkyl carbon chains. By incorporating double bonds, oxygen, nitrogen, or other heteroatoms, alternate retention and selectivity can be accomplished. These chemical interactions are generally not well understood or easily demonstrated, which can leave users frustrated and not using the phase to its potential.

In this study we set out to explore the retention mechanisms of the Restek IBD stationary phase. This proprietary phase offers unique selectivity by embedding a polar group into an alkyl chain which takes a positive charge under selected conditions. In addition to the traditional reversed-phase dispersive interactions, this phase may exhibit hydrogen bonding and anion-exchange interactions which aid in selectivity of specific analytes. Our focus in this presentation is on specific method parameters including temperature, and mobile phase acid strength and concentration and how these changes affect the selectivity of target analytes. By demonstrating the influence of specific method changes on this phase we aim to gather a better understanding of the interactions demonstrated by the IBD phase and its use as a HILIC or mixed-mode phase.

Keywords: Chromatography, HPLC Columns, Separation Sciences
Application Code: General Interest
Methodology Code: Liquid Chromatography
We will explore the hydrophobic-subtraction model and how the model relates to retention mechanisms in HILIC, mixed-mode and reversed phase techniques. Particular attention will be paid to the solutes they are most frequently used for. By utilizing the model, we will explore and expand upon the individual terms in order to understand the effects of each term in HILIC and mixed-mode retention. Examples from the model, as well as applied examples, will be used to demonstrate the mechanism for each term.

The selectivity and retention will be demonstrated on selected phases that were designed to accentuate selected terms. These fundamental interactions are the drivers in every liquid separation we perform, regardless of whether they are marketed as HILIC, mixed-mode, or reversed phase. Understanding, and in some cases accentuating, these interactions can lead to simpler method development which yield much more consistent methods and results.

Keywords: Chromatography, HPLC Columns, Liquid Chromatography, Method Development
Application Code: General Interest
Methodology Code: Liquid Chromatography
Separation Sciences: General Interest, Materials Science and Others

**Development of a Strategy for Scaling SFC Methods**

It is well known that for separations using CO2 as the principal component of a mobile phase, analyte retention factors are influenced largely by the mobile phase density and temperature. Because of the high compressibility of CO2 under standard operating conditions, the density can change significantly with changes in pressure (under isothermal conditions) with retention factors decreasing with increasing mobile phase density (pressure). In addition, the selectivity and resolution of the analytes may be impacted as they respond differently to the same changes in mobile phase density. This can present a challenge when attempting to transfer a method between different column configurations that involve changes in column length or stationary phase particle size, which in turn alters the pressure (density) profile for the separation. This is best exemplified when analytical scale separations are developed using sub-2-µm stationary phases, and then need to be scaled up to preparative SFC using 5 µm particle size stationary phases. The difference in the density profiles across the column, between the analytical and the preparative system, will lead to very different chromatography unless the scale-up procedure is guided by a systematic approach.

Here, we present a strategy for scaling SFC separations between various column configurations. This will be demonstrated for the transfer of methods between analytical columns of different configurations, and more interestingly, for a method developed under analytical SFC conditions with subsequent scale up to preparative SFC conditions. This ability enables the rapid screening of methods on the faster analytical scale, with the direct transfer of the final method to preparative chromatography, resulting in significant savings in time and mobile phases.

**Keywords**: Pharmaceutical, Prep Chromatography, SFC

**Application Code**: General Interest

**Methodology Code**: Separation Sciences
In order to perform faster gradient analyses by applying a high constant pressure (cP), Agilent Technologies (Waldbroon, Germany) revived the concept of volume-based (VB) gradient. The properties of VB gradients guarantee an excellent injection-to-injection repeatability of the elution volume despite the unavoidable fluctuations of the flow rate over time. This remains true under steady thermal conditions.

In contrast, columns are not isothermal when fast gradient analyses are performed with columns packed with fine particles. The temperature distribution may not be reproducible from one analysis to the next one. Often, the next injection is made before the column has time to return to the initial temperature of the previous run. Therefore, the repeatability of retention volumes can be randomly affected. This problem can be alleviated if the analyst operates gradients under a quasi-stationary state temperature (QSST) regime.

This presentation discusses a method that yields reproducible retention times and peak profiles when dealing with unsteady thermal conditions. The QSST regime consists in determining the flow rate profile that should be applied during the three gradient steps (the run, the wash, and the re-equilibration), in order to keep constant the temperature distribution across the column during the gradient runs. The ideal flow rate profile is provided by the simultaneous solution of the energy balance and the permeability equations. An experimental validation of this approach is provided by the results of measurements of the outlet eluent temperature for 20 consecutive gradient runs made at very high pressures and flow rates (400 to 600 bar, 3 to 5 mL/min), using different flow rate profiles. Finally, the advantages for analysts of running QSST regime gradients are demonstrated by comparing the repeatability of the elution volumes obtained with this method and those provided by the classical fast cF and cP gradients.

Keywords: Chemical, HPLC, HPLC Columns, Liquid Chromatography
Application Code: Drug Discovery
Methodology Code: Liquid Chromatography
Separation Sciences: General Interest, Materials Science and Others

An Ultimative Axial Compression Steel Column

This high quality column hardware combines benefits for every HPLC user who needs highest performance as well as robust and long lasting columns in preparative scale LC for quality control. The columns realize axial compression of the bed to get long lasting columns with excellent performance. Available diameters are 20, 30, or 50mm with 30, 150, or 250mm lengths. The columns provide enhanced operability using one adjustable end fitting, optimum packed bed density and stability in a universal design, and can be used with virtually any media. The effect of band broadening is demonstrated before and after axial compression.

Keywords: Chemical, HPLC Columns, Laboratory, Liquid Chromatography

Application Code: Quality/QA/QC

Methodology Code: Liquid Chromatography
Previously, spectroscopic analyses such as Raman, FTIR, or Near-IR were done in analytical laboratories on collected samples. Sample handling, analysis, and data reporting could result in a delay between a sample result and a decision that needed to be implemented, the release of raw material for manufacturing, for example. Optimization and miniaturization of optoelectronic devices have allowed the development of several portable technologies that can be brought to the point of sampling, such as in the field or the warehouse. This can result in improved testing cycle times and efficiency enhancement that is good for the analytical consumer. The hazard is once portable technology is adopted, test method development can be hindered by the tendency to apply a single technique to all problems rather than selecting an application-appropriate technology.

There is also confusion regarding when it is best to apply certain spectroscopy technologies. In specific areas, there exists an overlap, with multiple technologies being suitable to solve certain problems, but each technique has its areas of strength and weakness. This presentation compares the portable instrumentation technologies Raman, Near-IR, FTIR, and XRF and provides guidance for the selection of applications specific tools.

Keywords: Identification, Materials Characterization, Portable Instruments, Spectroscopy
Application Code: General Interest
Methodology Code: Portable Instruments
Raman spectroscopy is a powerful technique for material identification. The technique is sensitive to primary and higher ordered molecular structure features such as crystal structure and conformation. The relationship between excitation laser wavelength and intensity of Raman scattering results in a higher energy, shorter wavelength excitation laser scattering photons with greater intensity than a lower energy, longer wavelength laser. Comparison of instrument components includes a combination of both Raman scattering intensity and detector sensitivity for each excitation wavelength. A superficial comparison of instrument components might lead to the false conclusion that a shorter wavelength 785 nm Raman system is superior to a longer wavelength 1064 nm Raman system.

However, Raman scattering is a relatively weak effect compared to fluorescence, a competing emission phenomenon. When fluorescence occurs, the Raman signal is easily obscured. Trace levels of a fluorescing substance will emit more light at a greater intensity than the Raman photons, preventing desired measurement, and the likelihood of fluorescence interference increases at shorter excitation wavelengths. The best way to decrease the likelihood of fluorescence interference is to measure Raman spectra using a longer wavelength excitation laser. When using a 1064 nm laser, for example, good quality spectra can be obtained, although weakly scattering materials may require longer integration times and signal averaging to decrease noise. Compared with 785 nm excitation, measurements taken at 1064 nm excitation can more often be performed without confounding by fluorescence interference.

Keywords: Identification, Materials Characterization, Portable Instruments, Raman
Application Code: General Interest
Methodology Code: Portable Instruments
Detection of Emerging Contaminants in Water by a Displacement Assay Based on High-Performance Affinity Chromatography

Recent reports and research by the World Health Organization have discovered pharmaceuticals and other related contaminants in various types of water sources, especially recycled or reclaimed water. This is a growing concern worldwide since many regions commonly use reused or recycled water for irrigation. The purpose of this study was to explore the use of high-performance affinity chromatography as a tool for rapidly screening for common emerging contaminants found in reused or recycled municipal wastewater. A displacement assay was constructed by using a fluorescent labeled analog of the drug phenytoin and an immobilized bovine serum albumin (BSA) affinity column. BSA is a serum transport protein found in cattle that has a series of general binding sites that are capable of binding to various pharmaceuticals and hormones, as well as some pesticides. Warfarin, carbamazepine (widely found in wastewater), and L-tryptophan were used as the model chemicals and binding probes for this assay. All of the compounds were found to displace the labeled phenytoin when applied to the BSA column and provided a signal within a few minutes of sample application. The information provided by this study indicates that this approach can be used as a screening tool to detect emerging contaminants in environmental water samples. The use of such columns with LC/MS/MS to examine water contaminants that are captured by the BSA column is also being examined.

Keywords: Environmental/Water, HPLC
Application Code: Environmental
Methodology Code: Liquid Chromatography
Recent advances in web-based in-situ instrumentation will be presented using a new electrochemical system that is completely controlled using any web browser. This system can control and collect data from a variety of marine platforms using radio, satellite or cellular communications. Field data from a variety of applications will be presented. Additionally, a unique web-based micro-observatory system will show data collected from a fresh water system and show the relationship between diurnal and photosynthetic cycling. This system simultaneously collects calibrated data from a variety of sensors and serial instruments, resulting in very robust and easy to use system.

Keywords: Electrochemistry, Environmental Analysis, Instrumentation, Water
Application Code: General Interest
Methodology Code: Other (Specify)
In parallel segmented flow chromatography (PSF) the flow of the mobile phase is dynamically managed as it passes through the HPLC column, to eliminate wall effects and minimise solute band broadening, therefore maximising the performance of the column. This is achieved by separating the central flow region of the bed from the peripheral or wall flow region using an annular frit design, and a multi-channel end fitting.

The practical advantages of using PSF have been described in the literature for fully porous [1,2] and monolithic materials [3]. In this paper we report the results of a study comparing the performance of two column configurations (standard and PSF) where the columns are packed with solid core particles. Peak efficiency, peak asymmetry and peak height are monitored. Significant improvements are observed, with PSF providing up to 20% higher efficiencies, 5-10% improved peak height and more symmetrical peaks.

References:
The synthesis and characterization of a peptide-based chiral ionic liquid, Glycine-L-histidine bis(trifluoromethane)sulfonimide (Gly-L-His-NTF$_2$) and a series of amino acid-based chiral ionic liquids including, L-phenylalanine tert butyl ester bis(trifluoromethane)sulfonimide (L-Phe t-bu O NTF$_2$), L-Phenylalanine benzyl ester bis(trifluoromethane)sulfonimide (L-Phe Be O NTF$_2$), L-Phenylalanine methyl ester bis(trifluoromethane)sulfonimide (L-Phe Me O NTF$_2$), and L-Phenylalanine ethyl ester bis(trifluoromethane)sulfonimide (L-Phe Et O NTF$_2$) is reported. The CILs were prepared following a literature procedure (Tao G. H. et al, Chem. Commun. 2005, 3562.) where equimolar amounts of Glycine-L-histidine HCl, each of the individual L-Phenylalanine ‘alkyl’ ester HCls, and lithium bis(trifluoromethane)sulfonimide were dissolved in a minimal volume of water. The solution formed two layers, and the CIL layer was separated from the aqueous layer. The CILs were rinsed and the chloride impurity removed by extraction with dicholoromethane. The resulting CILs were dried under vacuum. $^{1}$H and $^{13}$C NMR spectroscopy was used to confirm the purity of the CILs. Analysis of the physico-chemical properties was evaluated using DSC, TGA and viscometry. The thermal decomposition temperatures of the CILs ranged from 260.07 °C to 385.34 °C. The melting points and glass transition temperatures of the CILs ranged from 69.56°C to 108.3 °C and -43.65°C to -51.29 °C, respectively. Variations were observed in the viscosity of the L-phe ‘alkyl’ ester NTF$_2$ CILs as the alkyl spacer changed. The CILs were found to be highly fluorescent. The fluorescence spectral behavior of the neat L-phe ‘alkyl’ ester NTF$_2$ CILs was different when excited at the same wavelength and the emission spectra shifted to longer wavelengths as the excitation wavelength was increased.

Keywords: Amino Acids, Characterization, Chiral, Chiral Separations
Application Code: Materials Science
Methodology Code: Separation Sciences
Active Flow Technology (AFT) is a new HPLC column platform in which the flow of the mobile phase as it passes through the column can be dynamically managed to eliminate wall effects, minimise solute band broadening, maximise signal response or maximise theoretical plates has been introduced. The concept of this technology is to counter the lack of radial homogeneity that is associated with packed columns, in particular near the walls where the rigid structure the column housing does not facilitate a homogenous packing arrangement for solid spherical particles. The effects that radial inhomogeneity has in terms of column performance have been well described by numerous researchers.

The use of this technology has been solely applied to spherical particles packed into a stainless steel tubes. With this configuration of hardware and using the active flow technology, it has been demonstrated that the efficiency and the signal intensity can be improved. To date this technology has not been coupled to monolithic stationary phases, which suffer from the same physicalities in terms of flow profile as with a column packed with spherical particles. This presentation will address this, presenting data from a monolithic column and two different configurations of the AFT. It will be demonstrated that improvements in chromatographic efficiency of up to 50% can be achieved. By using the same hardware at the front end of the column it is possible to create a virtual column, where similar performance benefits can be realised, but also with an increase in sensitivity. Data will be presented from this alternative AFM configuration which demonstrates an increase in sensitivity of greater than 100%.

Keywords: Chromatography, HPLC Columns
Application Code: General Interest
Methodology Code: Liquid Chromatography
Separation Sciences: General Interest, Materials Science and Others

The Reproducibility of Constant Flow and Constant Pressure Chromatography: Time vs. Volume Based Chromatograms

Conventional HPLC and very high pressure liquid chromatographs (VHPLC) are operated under a constant flow rate and chromatograms plotted as a function of time eluted. Recently an instrument capable of constant pressure operation was designed. The chief advantage of performing constant pressure gradient separations is mainly a reduction in time for analysis. If column permeability decreases during constant flow operation, the pressure limits of the column and instrument can cause the instrument to stop analysis mid run. This would be of concern if there was a long series of analysis being performed. Constant pressure chromatograms should be plotted in units of volume eluted to correct for discrepancies in the flow rate from run to run. Several methods of delivering the gradient were examined to assess the reproducibility of time and volume based chromatograms obtained from the instrument. These methods include: constant flow rate conditions, constant pressure conditions, very high constant pressure conditions, and programmed flow constant pressure conditions. All experiments were conducted using a prototype instrument capable of constant pressure operation. A solvent gradient of 50-95% (water/acetonitrile) was delivered over analysis times of approximately 2 minutes to fully resolve the components of a nine component sample for all the discussed methods. A series of 6 separations were conducted for each of the methods, with sufficient post-run times to bring the column to the same temperature for the start of each separation. Results show that using a constant pressure gradient and a programmed flow constant pressure gradient can reduce the analysis time of separations by 16-22%, with respect to constant flow operations. The advantage of a programmed flow constant pressure gradient is that chromatograms can be highly reproducible when plotted in both time and volume units; while reducing analysis time.

Keywords: High Throughput Chemical Analysis, HPLC
Application Code: High-Throughput Chemical Analysis
Methodology Code: Liquid Chromatography
To improve molecular recognition property, the molecular imprinting was conducted on the surface of the sol-gel particles. The particles were prepared using TEOS and 3-(trimethoxysilyl)propyl methacrylate (MPS) by the little modified Stoeber method. The mixture that particles were mixed with template, MMA, AA, and EGDM as a cross-linker, was polymerized by dropping it slowly into hot water of 75 – 80 C. Then the polymer was extracted in dioxane until the sol-gel particle free polymer was removed, and not to detect template by UV spectrometer. The sorption characters of 2-ethylaniline imprinted particles were investigated on the ethylaniline isomers in the aqueous media. With the Scatchard plot, the selectivity was considered how the correlation are existed between them.

Keywords: Analysis, Separation Sciences
Application Code: Nanotechnology
Methodology Code: Separation Sciences
Abstract

In the field of chromatography, there has been much attention given to hybrid materials that have two aspects of inorganic and organic characteristics; high efficiency, and high mechanical and chemical stability. Recently, we have developed a hybrid silica based UHPLC and HPLC column named “YMC-Triart series”. YMC-Triart series is based on multilayered organic/inorganic hybrid particles with 1.9 [micro]m, 3 [micro]m and 5 [micro]m diameters, and available in C18, C8, Phenyl, PFP and Diol-HILIC chemistries. Nowadays, various chemistries bonded on a hybrid material are available in the market. Especially, unique separations on hydrophilic compounds analysis with several chemistries other than C18/C8 are reported. In this poster, we focus on separation characteristics of Triart PFP and Triart Diol-HILIC on highly-polar biologically active substances. We will introduce performance and advantages of those phases through some examples of hydrophilic compounds analysis.

Keywords: Amino Acids, Biological Samples, HPLC, Medical
Application Code: Biomedical
Methodology Code: Liquid Chromatography
Abstract Text

One important trend in modern chromatographic science is the reduction of size of the complete separation system. Such developments may be important for several reasons, including enhanced sensitivity, reduction in the amount of sample required, minimizing the amount of solvents used and waste generated. The limiting factor, however, is often the volume of the column. Modern chromatographic analyses employ 2.1mm columns. The present efforts focus on extending the routinely usable range to 1.0mm and 300µm columns to obtain 77% and 98% reductions, respectively, in sample amount requirements. The benefits of size reduction can be further enhanced by using UPLC technology employing columns packed with sub-2µm particles. The effective application of these dimensions of columns requires migrating methods among sizes while preserving chromatographic quality. Three main instrument characteristics must be optimized, specifically, solvent delivery, sample introduction, and detection. The solvent manager was evaluated at flow rates appropriate for the three UPLC column diameters. A simple translation of flow rates was developed to give constant linear velocity that preserves resolution. This translation was extended to account gradient operation including the effect of system volumes and time offsets. The sample manager has been tested for the interaction among mass loaded, volume loaded, sample diluent, and retentivity. This interaction has been thoroughly measured. Finally, TUV detector cell effects on the small volume peaks has been determined and optimized. These instrument characterization experiments facilitate the use of different dimension columns while preserving resolution and selectivity in UPLC analyses.

Keywords: Capillary LC, Chromatography, HPLC, HPLC Detection
Application Code: Other (Specify)
Methodology Code: Microfluidics/Lab-on-a-Chip
HILIC and Mixed-Mode Retention of the Pentafluorophenyl Propyl (PFPP) Stationary Phase

HILIC and Mixed-Mode are terms originally used to describe a specific chemical interaction or set of interactions in liquid chromatography. Recently, however, these terms have been more frequently used to describe LC columns offering retention mechanisms that vary, or are orthogonal, to a C18. These columns generally consist of a bonded phase which contains a chemical moiety other than, or in addition to, alkyl carbon chains. By incorporating double bonds, oxygen, nitrogen, or other heteroatoms, alternate retention and selectivity can be accomplished. These chemical interactions are generally not well understood or easily demonstrated, which leaves users frustrated and not using the phase to the fullest potential.

In this study we set out to explore the retention mechanisms of the PFPP stationary phase. This phase offers unique selectivity by incorporating strongly electronegative fluorine atoms on a phenyl ring. In addition to the traditional reversed-phase dispersive interactions, this phase may exhibit cation-exchange and pi-pi interactions which aid in selectivity of specific analytes. Our focus in this presentation is on method changes including temperature, mobile phase, and acid strength and concentration and how these changes affect the selectivity of target analytes. By demonstrating the influence of method changes on this phase we aim to gather a better understanding of the interactions provided by the PFPP phase and its use as a HILIC or mixed-mode phase.

Keywords: HPLC Columns, Separation Sciences
Application Code: General Interest
Methodology Code: Liquid Chromatography
Back-scattering interferometry (BSI) is an unusually sensitive (zeptomole) universal detection platform with a simple optical train, consisting of a coherent source, microfluidic chip and detector. A unique multi-pass configuration allows BSI to transduce minute changes in the refractive index of a solution into equilibrium binding constants over a range of at least six orders of magnitude, without any labeling and in free-solution. It also allows analyte quantification at femtomolar concentrations in complex milieu. This overview teaches how BSI works and shows it is a tool that can be widely used across scientific disciplines. Examples of the enabling characteristics of BSI presented include the quantification of binding affinity for antibody-antigen, protein-ion, protein-protein, and aptamer-protein interactions. Respiratory virus RNA detection investigations further illustrate that BSI measures conformational changes and can be a sensitive detector. Next, DNA hybridization determinations and sugar-lectin binding studies illustrate that BSI is not limited by mass sensitivity as with other bio-sensors, that it can lend insights into multivalent interactions, and can quantify the influence of labels and/or surface immobilization. Another attribute of this unique interferometer, complex matrix compatibility, is illustrated through the direct quantification of binding affinities for membrane-associated protein drug targets in native bilayer environments, cell extracts, and serum. Membrane-protein studies with BSI have allowed characterization of the molecular mechanism of inhibition for several classes of species including the lipid receptor Lysophosphatidic Acid and the enzyme Cyclooxygenase II. Work on hydrogen bonding studies in non-aqueous media will show more general applicability of BSI, while investigations toward near-patient diagnostics and biomarker validation provides evidence it is a quantitative sensor with significant advantages in biomarker validation.

Keywords: Bioanalytical, Biological Samples, Protein
Application Code: Bioanalytical
Methodology Code: Other (Specify)
Backscattering interferometry (BSI) is an emerging analytical technique that detects changes in refractive index on the order of 10-7 RI units based on the binding interactions of two or more species. This talk will focus on recent work in our group in the study of 3 systems: 1) Oligonucleotide hybridization, 2) Detection of acetylcholinesterase-inhibitor interactions, and 3) detection of hydrogen bonding in non-aqueous solvents.

To study oligonucleotide hybridization, a direct comparison of surface-immobilized and free-solution methodologies was carried out using BSI as the sensing platform. Results from these experiments show a clear perturbation due to immobilization, with K values nearly 50% higher than free-solution experiments. While the K values for the surface-immobilized experiments were higher than free-solution, both data sets exhibited the same trends.

The interaction of a series of known and novel inhibitors of acetylcholinesterase (AChE) was examined using BSI. At the lowest limit, BSI can detect AChE-inhibitor interactions at 100 pM and avoids false positives with acetylthiocholine substrates seen in colorimetric techniques. Additionally, the experiments can be carried out in free-solution without the need for fluorescent labels.

In the last portion of the talk, the application of BSI for the study of small-molecule interactions in organic solvents will be presented. Intermolecular interactions involving hydrogen bonds are responsible for catalysis and recognition. Traditional methods used to study hydrogen-bonding interactions are generally limited to relatively large volumes and high substrate concentrations. These studies show that BSI provides a microfluidic platform to study these interactions in non-aqueous media at mM to nM concentrations in pL volumes by monitoring changes in refractive index.

Keywords:
- Biopharmaceutical, Chemical
Application Code:  Bioanalytical
Methodology Code:  Physical Measurements
Abstract Text

Molecular interactions govern biology, human health, disease and pharmacological efficacy of therapeutics (both small molecules and biologics). Therapeutic dose-response relationships are predicated upon accurate measures of drug binding interactions to a target at the site of action. Clinically relevant measurements are especially problematic since target proteins reside in complex physiological environments, such as biological fluids or tissue microenvironments as soluble and/or membrane-bound forms. This talk will describe how Back-scattering Interferometry (BSI) has been used to solve the problem of measuring physiologically-relevant affinity that is used to predict clinical dose and efficacy.

Keywords: Biological Samples, Biosensors, Membrane, Quantitative
Application Code: Pharmaceutical
Methodology Code: Sensors
Lung cancer is the number one cause of cancer related mortality in men and women in the US and worldwide. Lung cancer kills more men and women than colorectal, prostate, and breast cancer combined. Most cases are diagnosed at an advanced stage when cure is no longer an option. Early detection has the promise of revolutionizing the outcome of this deadly disease. There is no biomarker currently in practice that is known to reduce lung cancer related mortality. Biomarker quantification is critical to realizing the goals of personalized medicine which promises to enable early disease detection, diagnostic staging and therapeutic efficacy monitoring. Biomarker validation continues to be a bottleneck to clinical translation. Backscattering interferometry (BSI) could aid in overcoming this restriction. We compared BSI to ELISA and validated the platform for the quantification of two candidate biomarkers for lung cancer in spiked serum and actual patient samples. We demonstrated that BSI can enable rapid biomarker validation in complex, volume constrained samples. A BSI dose-response curve was constructed for Cyfra 21-1 and galectin-7 (LGALS7) by incubating increasing concentrations of recombinant protein in spiked human serum with 100 ng/ml of antibody. An ELISA dose response curve was similarly constructed according to the manufacturer’s recommended procedure. The lower limit of detection for LGALS7 was determined to be 0.5 ng/ml for ELISA in serum and 13 pg/ml for BSI. The lower limit of detection for Cyfra 21-1 was 1.0 ng/ml and 240 pg/ml for ELISA and BSI respectively. In conclusion, BSI correlates well with ELISA, provides significantly lower detection limits, more rapidly, with greater ease, and significantly less sample consumption.

Funding support: EDRN UO1 CA152662

Keywords: Analysis, Biological Samples, Biomedical, Immunoassay
Application Code: Biomedical
Methodology Code: Other (Specify)
The measurement of binding interactions to polyvalent scaffolds such as polymers, dendrimers, proteins, and nanoparticles is often done with tools such as ELISA that are highly sensitive to polyvalent effects. It is therefore difficult to discriminate between affinity and avidity in many of these cases. Backscattering interferometry (BSI) detects the intrinsic changes in solvation that occur in each binding event, and so provides a unique label-free window into molecular interactions. The results of BSI measurements on the binding of polyvalent virus-like particles to antibodies, other proteins, and cells will be described, as well as studies of the binding of encapsidated aptamer RNA with small molecule ligands.

Keywords: Bioanalytical, Biomedical, Biospectroscopy
Application Code: Bioanalytical
Methodology Code: Biospectroscopy
Development of diagnostic methods for rapid and sensitive identification of biomedical pathogens is essential for the advancement of therapeutic and intervention strategies necessary to protect public health. Current diagnostic methods, e.g. culture, isolation, PCR, antigen detection, and serology, are often time-consuming, cumbersome, or lack sensitivity.

We have investigated several different nanoparticle platforms for surface-enhanced Raman (SERS)-based identification and classification of pathogens. These platforms included metal colloids, nanosphere arrays, OAD nanorod arrays, and layer-by-layer nanoparticle assembly. The current talk will address the development of spectroscopic methods for pathogen detection based on these nanostructured SERS platforms. Examples will include the use of click chemistry for building carbohydrate microarrays as well as the detection of Mycoplasma pneumonia in simulated and true clinical specimens. We will present multivariate analyses that indicate it is possible to identify, differentiate and classify pathogens based on their intrinsic SERS spectra, even down to the individual strain level.

Keywords: Bioanalytical, Biomedical, Chemometrics, Surface Enhanced Raman
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
The complex and dynamic nature of biological systems necessitates rapid, label-free sensing platforms. Combining the chemical stability of mesoporous silica core shell nanomaterials and the chemical sensitivity of surface-enhanced Raman scattering (SERS)-active gold core nanoparticles presents an optimal platform for stable and reliable SERS sensing. Herein, novel core-shell mesoporous silica nanosensors were fabricated to encapsulate different morphologies of gold nanoparticles, including nanorods and nanosphere aggregates. The nanosensors were extensively characterized using several microscopic and spectroscopic techniques, and then SERS spectra were captured from analytes presented in a matrix of whole blood. The presented data will include considerations of sensitivity, selectivity, and stability of this novel core-shell sensing platform.

Keywords: Biosensors, Modified Silica, Nanotechnology, Surface Enhanced Raman
Application Code: Bioanalytical
Methodology Code: Vibrational Spectroscopy
Surface enhanced Raman spectroscopy excited at 785 nm is found to be a sensitive probe of the metabolic products of bacterial and human cells. Cells removed from the human body undergo characteristic in vitro robust biological activity whose detection can be exploited for a number of biomedical, diagnostic and forensic applications. In particular, the degradation products resulting from energy depletion in bacterial cells provides a unique SERS signature that can be both species and strain specific. An in situ grown, This methodology is being developed for diagnosing blood and urinary track infections. Robust antibiotic specific bacterial identification can be accomplished with this technique.  

785 nm excited SERS spectra of human blood and red blood cells (RBCs) are obtained and are due to blood plasma and hemoglobin, respectively and may be exploited for several biomedical purposes. The SERS spectrum of whole human blood changes dramatically over the course of ~ 24 hours. The spectrum of stored blood becomes nearly dominated by hypoxanthine, a metabolite of pure degradation, over this period of time due to it’s release into blood serum from blood particles. Tumor cells are well-known to exhibit high metabolic rates compared to normal, non-pathogenic cells. Again, characteristic SERS vibrational signatures due to molecules like adenine, hypoxanthine and NADH appear over the course of several hours from single cancer cells. Thus SERS may provide a procedure 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. Finally, the use of SERS for trace detection and identification of human body fluids such as blood, semen, vaginal fluid and saliva will be described. The effects of human cell metabolism in these in vitro fluids can be observed and provide a novel methodology for ultrasensitive forensic identification at crime scenes.
Based on the local optical fields of metal nanostructures, SERS nanoprobes and labels can be constructed and applied in cells and biomaterials. In particular, SERS will be discussed in the context of applications in complex bioenvironments. SERS can be utilized for the analysis of cellular samples of plant and animal origin which are several tens to hundreds of microns in size [1]. There, nanoparticles of gold and silver are used to generate the enhanced local optical fields in which SERS takes place. In experiments with live cells, metal nanoparticles are transferred into the cell, and depending on their size and surface properties, can be directed to different cellular compartments, from where they can deliver different types of information. Plasmonic nanoprobes enable vibrational spectroscopic imaging with improved sensitivity and lateral resolution. As will be discussed for hemoglobin and red blood cells, the nano-bio-interaction may have serious consequences for biomolecular structure. [3]
The fabrication of a 3-D, homogeneous and highly dense gold nanorod (AuNR) assembly on electrospun polycaprolactone (PCL) fibers using electrostatic interactions as the driving force has been successfully carried out. Specifically, decoration of a poly(sodium 4-styrenesulfonate) (PSS) layer onto the AuNRs imposed negative charges on the nanorod surface and the interactions between PSS and the AuNRs were investigated using attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR). Positive charges on the PCL fibrous substrate were established via the polyelectrolyte layer-by-layer (LBL) deposition, which was investigated using multiple characterization techniques. Driven by the attractive electrostatic interaction, immobilization of AuNRs on the PCL fibers was initiated upon substrate immersion and the kinetics of the immobilization process were studied using UV-Vis spectroscopy. Electron microscopy characterization on the AuNR/PCL nanocomposite fibers revealed a uniform AuNR coating on the fiber surface with the immobilized AuNR density being high enough to provide full surface coverage. By using both 4-mercaptopyridine (4-MPy) and Rhodamine 6G (Rh6G) as the probe molecules, the performance of the AuNR/PCL fibers as a surface enhanced Raman scattering (SERS) substrate was investigated. The 3-D nanocomposite fibrous mesh allowed detection at concentrations as low as 10^-7M of the probe molecule in solution and exhibited excellent reproducibility in the SERS measurements.
Monoclonal antibodies (MAbs) are therapeutic proteins with important applications in many disease areas. The systematic and phase dependent characterization are critical to develop safe and efficacious drugs according to regulatory guidelines. Different modes of chromatography are the key approaches to support these characterization efforts. In this presentation, I will describe the finding of a unique stop codon mutation by characterizing unknown PR-HPLC peptide peaks for pre-IND clone screening. In addition, I will also present the detailed characterization of chromatography unknowns for a late state development project prepared for BLA filing. These examples indicate chromatography separations are indispensable tools to understand the characteristic of antibodies and reveal novel antibody modifications.
With the recent advances in analytical technologies, along with wide implementation of QbD, a comprehensive analytical strategy to support biologics and biosimilar products have become imperatively important. When developed in connection with critical quality attributes and design space, a strong analytical platform not only is key to understanding the products and manufacturing processes, it also can effectively guide future process changes and transfer by conducting comprehensive comparability studies. In this presentation, we will discuss the steps towards development of a comprehensive analytical strategy to support different stages of biologics development. We will also discuss the pros and cons of selective analytical platforms in order to provide practical guidance to analytical development.
Therapeutic proteins have become important class of medicines for treating a variety of human diseases and conditions. Unlike small-molecule drugs, structure of which can be verified through established analytical testing and for which compendial methods are generally available, large-molecule therapeutics not only have no commercially available international reference standards, but also lack standardized methods of their analysis. By the nature of these products, biotherapeutics are large in size and often decorated by multiple co- and post-translational modifications. Such added complexity necessitates application of multiple orthogonal methods and drives the development of new and improved state-of-the-art technologies to be used for characterization. Undoubtedly, improved analytical methods, and specifically applications of mass spectrometry, in the past two decades, not only improved better understanding of these molecules and their biological mechanisms of action, but also insured safety to patients, consistency of production and addressing increasing regulatory expectations.

This talk will focus on applications of mass spectrometry for characterization of biotherapeutic drugs. Typical applications of mass spectrometric and hyphenated tools to production monitoring, process optimization and product characterization will be discussed. Attention will be paid to what aspects of biotherapeutic need to be characterized in order to demonstrate it is a well characterized biologic and differentiating it from under- or over-characterized product.

Keywords: Biopharmaceutical, Liquid Chromatography/Mass Spectroscopy, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Although reversed phase is an extremely popular retention mode when it comes to biopharmaceuticals, ion-exchange continues to be widely used in the discovery characterization and in quality control. Here we describe the latest research results concerning the development of new high-performance ion-exchange phases designed specifically for the analysis of biotherapeutics. Included will be a discussion of stationary phase architectures as well as information about newly developed materials with advanced properties. In addition, this work will include a comparison of the utility of ionic strength gradients versus pH gradients in the characterization of monoclonal antibody charge variants.
Analytical support surfaces made from fibers with submicron diameters, nanofibers, have higher surface/volume ratios and higher porosity than supports produced from larger fibers, paper or films and can enable processes including filtration, liquid transport, chemical binding and mixing in analytical systems. Nanofiber membranes for analytical applications have been created by the electrospinning process. The flexibility of this process has allowed creation of materials with surface functions including hydrophilic, hydrophobic, positively charged, negatively charged, anti-fouling, conducting and piezoelectric. Additionally, specific active molecules including biotin and proteins and functional nanoparticles have been incorporated into the fibers. Electrospinning creates nanofibers by applying a high voltage to a polymer solution. A fine jet of material is pulled from the solution and follows a whipping path, stretching and solidifying until it lands on a grounded collector. The collector can be designed to collect fibers in patterns ranging from aligned arrays on a microfluidic chip to yards of nonwoven fabric. To address the particular challenge of creating hydrophilic, non-water soluble nanofibers, we have created nanofibers from Polylactic acid/Polylactic acid-b-Polyethyleneglycol blends via thermodynamically driven phase separation during the spinning process. Increasing the hydrophilicity of the fibers increases capillary driven flow through the nanofiber membrane. Bovine serum albumin and streptavidin protein have been added directly into these nanofibers during the spinning process to create active capture sights for use with liposome based sandwich assays. All aspects of the nanofiber membrane structure and properties are controlled by optimizing the spinning solution, process variables and collector design. Methods for producing nanofibers with this broad range of functionality and characterization of the nanofiber membranes will be presented.

Keywords: Materials Science, Membrane, Nanotechnology, Polymers & Plastics
Application Code: Materials Science
Methodology Code: Other (Specify)
Nano Fiber-Based Biosensors for Integrated Sample Preparation

Microfluidic biosensors, labs-on-a-chip and lateral flow assays for the detection of viable organisms, toxins, and clinically relevant markers have been successfully developed in our research group including analytes such as B. anthracis, C. parvum, dengue virus, E. coli, S. pyogenes, cholera toxin, CD4+ T-lymphocytes, thrombin and myoglobin. Biodetection is accomplished by taking advantage of optimal recognition elements including DNA probes, aptamers, antibodies, gangliosides; and in most cases ultra-sensitive detection is afforded via liposome signal amplification. The fluorescence, colorimetric or electrochemical biosensors are typically paired with sample preparation to enable rapid and inexpensive detection direct from the sample.

Recently, we initiated the study of electrospun nanofibers and their potential to enhance bioassays in paper-based lateral-flow assays (LFA) and in polymer-based microfluidic devices by adding functionalities to the formats otherwise not available. In the case of the LFA format we successfully demonstrated the de novo fabrication of nanofiber-mats as membrane material enabling immobilization of biorecognition elements, adding novel surface chemistries and preventing non-specific binding without the use of blocking reagents.

Nanofiber-enhanced microfluidic devices provide additional degrees of freedom for bioassay designs as nanofibers with various surface chemistries are electrospun into distinct locations in the microfluidic channels. As the resulting fiber mats can be of varying density and size and hence generate a 3D-structure within the channels intimate contact with the sample is guaranteed throughout the channel volume. Current investigations study these systems for sample preparation, as mixers, and as concentrators where, for example, a concentration of E. coli cells by a factor of 20,000 has already been demonstrated.

Keywords: Bioanalytical, Biosensors, Lab-on-a-Chip/Microfluidics, Sample Preparation
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Traditionally, a key component of trace analytical methodology has been slow, labour-intensive sampling and sample preparation steps. We are actively pursuing solvent-free, “green” approaches, which can integrate both sampling and sample preparation steps. The effort leads to the high throughput laboratory determination as well as convenient on-site and in-vivo analysis. This presentation we will focus on fiber based devices, which accomplishes this objective. They will include hollow fiber membranes, needle traps and SPME devices. Fundamental component of our research is to increase understanding of the extraction processes by developing mathematical models including using COMSOL program and verifying them using experimental data. This approach allows us to develop convenient extraction devices, optimum geometries, effective methods, and appropriate calibration procedures. The major new direction includes developing selective high surface area sorbents for biomarker quantification including proteins. For example, in the recent project we have developed Apt-SPME probes by covalently immobilizing 5-NH2-modified anti-thrombin DNA aptamer on the surface of electrospun poly(acrylonitrile-co-maleic acid) (PANCMA) probes, which exhibited highly selective capture, good binding capacity, high stability and good repeatability based on the recovery results of thrombin.


Keywords: Calibration, Extraction, Sampling, SPME
Application Code: Biomedical
Methodology Code: Sampling and Sample Preparation
Integration of Paper Microfluidic Methods for Detection of Infectious Diseases for Low Resource Settings

Under support of NIH NIAID, our lab leads a team developing a low-cost method for high-sensitivity protein-binding assays for point-of-care detection of infectious disease (initial target influenza). The team consists of UW, Seattle Children’s Hospital, PATH, and GE Global Research. Under support of DARPA DSO, we are developing a multiplexable autonomous disposable nucleic acid amplification test system for the same purpose (initial targets Staphylococcus aureus and influenza). The team is the same, plus Epoch Biosciences. Both systems are based on novel uses of inexpensive paper and paper-like membranes. Both are designed to be instrument-free fully-disposable diagnostic platforms capable of detecting multiple pathogens. The designs allow sample-to-result testing anywhere, and will be simple enough for untrained users.

Both prototypes are designed to detect pathogens from a nasal swab, and share the use of a number of novel paper-fluidic tools. Recent progress on the protein binding assay includes development of de-novo proteins for viral protein capture, methods for analyzing their association onto papers, and their controlled release from the substrates. In the nucleic acid assay, we have demonstrated the detection of Staphylococcus aureus using an isothermal amplification of characteristic bacterial DNA sequences. The prototype integrates 1) lysis of the nasal swab sample in a tube, 2) isothermal amplification of the DNA obtained, and 3) detection of the amplified target using colored labels on a lateral flow strip.

Keywords: Bioanalytical, Biological Samples, Biomedical, Lab-on-a-Chip/Microfluidics
Application Code: Biomedical
Methodology Code: Microfluidics/Lab-on-a-Chip
Fibrous stationary phases have been of interest for many years, with primary drivers being their low cost, diversity of surface chemistries, and rapid mass transfer characteristics. The principle downfall to wider utilization has been the poor hydrodynamic throughput realized by “packing” fibers into a column structure. We have developed the use of capillary-channeled polymer (C-CP) fibers as versatile stationary phases for protein separations. C-CP fibers are melt-extruded with diameters on the order of 30-50 micrometers in a shape that has eight channels/legs that extend along the fiber axis. When placed in a column structure, the legs of adjacent fibers interdigitate, forming (essentially) a network of parallel, open channels. Thus, columns can have very high permeability, translating to very low back pressures, offering the possibility of use of high mobile phase linear velocities. In the case of macromolecules, this is a wasted capability in porous media. Fortunately, the C-CP fibers are effectively non-porous, so high linear velocities result in no van Deemter broadening penalties.

We describe here the use of C-CP fibers in the area of protein analytics, including fast liquid chromatography separations and high-selectivity extractions. As the fibers are extruded from simple polymers (polypropylene, polyester, and nylon), there is a wealth of basic surface chemistries that can be used to affect separations. More importantly, there is a rich toolbox of potential surface chemistries can be employed to create high levels of chemical selectivity. These include adsorption of ligands (e.g., protein A), covalent coupling (e.g., TCT chemistry), or a novel head group-modified PEG-lipid strategy. Examples will be given for each of these concepts, demonstrated first on a micropipette tip format, and then extended to columns. The combination of efficient fluidic movement, and diverse surface chemistries, make C-CP fibers highly versatile stationary phases.

Keywords: Bioanalytical, Liquid Chromatography, Proteomics
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Method Development Strategies for Two-Dimensional Liquid Chromatography

Selecting a Suitable Column for the Second Dimension in RPxRP

Comprehensive on-line two-dimensional liquid chromatography (LCxLC) can produce considerable 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 ($t_c^{2}$) and achieving fractional coverages ($f_{cov}$) close to 1. Typically $t_c^{2}$ is at an optimum between 12 and 21 s. We have also shown that using carbon clad columns in the second dimension ($D^{2}$) produces $f_{cov}$ ranging from 0.58-0.63 relatively independent of the choice of the first dimension ($D$) 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 $D^{2}$. The sample and $D$ columns were similar to those used in the prior study. The SB-C18 $D^{2}$ 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 $D^{2}$ are lower than previously observed, the carbon clad $D^{2}$ column increased the $f_{cov}$ values by a factor of 1.25 to 3 depending on the $D$ column used.
Two dimensional HPLC (2D HPLC) is a rapidly emerging tool for analyzing complex pharmaceutical samples. The product of the peak capacities combined with high resolution detectors is invaluable for high capacity samples (tryptic digests, stressed degradation, etc.). When combined with combinatorial/peak tracking software, the applications expand to detection of trace level impurities and sample enrichment through the use of fast orthogonal methods. While 2D HPLC offers an integrated high throughput approach for complex samples, the 1D instrument capacity of a laboratory and data management must be considered when implementing 2D HPLC. These considerations and their impact on both small and large molecule applications of 2D HPLC will be the focus of this presentation.
Ensuring patient safety and drug product quality is utmost priority and responsibility of pharmaceutical industries and regulatory agencies around the world. Analytical testing including appearance, identity and purity are performed to safeguard interest. Assay and related substances method usually performed using HPLC with diode array detection (DAD) and/or mass spectrometry (MS) detection is critical. Developing HPLC method of desired specificity and sensitivity can be challenging, as chemical components eluting in proximity of active pharmaceutical component (API) are usually similar and/or isomers limiting the use of DAD and MS. Further, levels are few orders of magnitude lower making it difficult to analyze. Pharmaceutical industry relies on manufacturing strategy, extensive column screening with different mobile phases/additives, stress samples to challenge/optimize the method. The diligence though adequate, cannot guarantee absence of potential co-eluting impurity(s). Given the stakes, it's surprising the industry is yet to embrace 2D-LC as an alternative. Possible explanations include lack of commercial instruments, inability to achieve comprehensive separation, baseline noise from switching valve limiting accessible area. Also, unlike biologics or polymeric samples, in pharmaceuticals repeated gradient in second dimension is not an option. Use of stepped, isocratic elution in the second dimension limits fractions transferred to secondary dimension.

The presentation will cover method development strategies for small molecules using automated 2D-LC-MS with similar/complementary phases in two dimensions, approaches to render selective regions of chromatograms into comprehensive separation. In addition, merits of heart-cutting 2D-LC over comprehensive 2D-LC for simultaneous, sequential, quantitative achiral-chiral analysis will be presented. Case studies highlighting importance of 2D-LC-MS in pharmaceutical analysis will be discussed.

**Keywords:** Chemical, Chiral Separations, Liquid Chromatography/Mass Spectroscopy, Pharmaceutical
Food products are very complex mixtures containing many nutrients of organic and inorganic nature. Their analysis may be directed to the assessment of food safety and authenticity, the control of a technological process, the determination of nutritional values as well as the detection of molecules with a possible beneficial or a toxic effect on human health.

From an analytical stand-point, one-dimensional liquid chromatography is widely applied to the analysis of food samples. Although such a method often provides rewarding analytical results, the complexity of many naturally occurring real-world samples exceeds the capacity of any single separation system. As a consequence, in the last two decades, the use of multidimensional chromatography (MD-LC) became mandatory whenever the complexity of the sample overwhelmed the resolution capability of any one-dimensional separation technique. To this regard, comprehensive (2D) techniques in which the whole eluate from the first dimension (1D) is fractionated onto the second dimension (2D) column turned out to be a valuable tool through careful selection of independent (orthogonal) separation modes. Moreover, from a detection viewpoint, MS and tandem MS techniques could be successfully employed for detailed structure elucidation through characteristic fragmentation pattern.

Method development methodologies including main instrumental/software aspects as well as selected applications in the field of food are here provided.

**Keywords:** Liquid Chromatography, Natural Products

**Application Code:** Food Science

**Methodology Code:** Liquid Chromatography
Abstract Text
Multidimensional chromatography has gained significant interest in the bioanalysis community in recent years. The major driving force behind its adoption is related to the ability of 2D-chromatographic methods to improve the separation of the analyte of interest from other sample components (matrix) in order to reduce suppression of the analyte signal. In this way, 2D-chromatographic techniques are responsible for increased sensitivities when compared to one dimensional LC methods using the same amount of sample. Two bioanalytical applications will be described covering identification and quantification of host-cell protein (HCP) impurities in protein biopharmaceuticals and a universal methodology for quantification of therapeutic mAbs in human serum.

In the case of the HCP assay, the therapeutic biopharmaceutical (a mAb) is reduced, alkylated and digested with trypsin. Peptides were separated by comprehensive two-dimensional RP/RP chromatography with high pH in the first dimension and low pH in the second dimension. Peptides from the second-dimension RP separation were detected and fragmented by a quadrupole time-of-flight mass spectrometer using alternating collision energy (low/high CE) MS scans. The data is searched against a database to identify HCPs in the mixture and to provide target peptides (and MRM transitions) for HCP quantification.

The development of a generic, fast and automated LC-SRM method for quantification of therapeutic mAbs in human serum using two dimensional 2.1 mm ID LC-SRM with heart cutting will be discussed. Peptides were separated by RP chromatography at pH 10 in the first chromatographic dimension. Only peptides used for trastuzumab quantification were transferred to the second chromatographic dimension undergoing an orthogonal separation at pH 2.5. After 2D chromatographic separation, the sensitivity of the LC-SRM assay was restored to 75% of the sensitivity observed in the absence of the serum digest matrix.

Keywords:  HPLC, Method Development, On-line, Peptides
Application Code:  Bioanalytical
Methodology Code:  Liquid Chromatography
We have previously shown that complex phenomena such as the development of secondary brain injury following acute severe traumatic brain injury can be detected and potentially understood by combining data from a range of real-time measurements in the brain tissue at risk from secondary injury (1). In this presentation we discuss the design, construction and first data from a new integrated clinical instrument specifically targeted at the detection of pathological 'secondary insults' to at risk brain tissue.

Chemical measurement is at the heart of the system. The molecular composition of the extra cellular space is sampled using an FDA approved micro dialysis probe. The dialyse liquid stream is then carried to the bedside analysis unit in either continuous flow or, for improved temporal response, using 30 nl droplets formed using FC40 fluorocarbon oil. Neurochemical analysis takes place at the patient bedside using electrochemical sensors and biosensors within a microfluidic analysis device. Potassium ion concentrations are measured using 300 [micro]m diameter valinomycin based ion-selective electrodes, 320 [micro]m integrated amperometric biosensors use used for levels of glucose and lactate in the microdialysate. These biosensors use an electropolymerised no-conductive film as a selectivity layer combined with PEG based enzyme entrapment. The microfluidic chip / sensor combination is built into a microfluidic circuit-board that also has computer controlled valves and micro-syringe pumps (LabSmith[registered]). This allows routine 5 point autocalibration under computer control. Custom instrumentation and event detection software that highlights adverse changes o the clinical care team then completes the instrument.

We believe that the new instrument allows the possibility of dynamic clinical management of such patents, and provides a robust framework to allow first clinical use of new biomeasurement technologies.


Keywords:  Biosensors, Electrochemistry, Lab-on-a-Chip/Microfluidics, Neurochemistry
Application Code:  Neurochemistry
Methodology Code:  Electrochemistry
More Than One Way to Skin a Cat: The Diversity of Analytical Tools for Chemically Mapping the Brain

In vivo microdialysis sampling has provided a wealth of valuable information on brain chemistry including insight into pathology, behavior, and pharmacology. The most common way microdialysis is used to collect fractions for analysis of neurotransmitter content by HPLC. Such methods typically only measure a few neurotransmitters at once. We have recently developed a UPLC-MS method that allows measurement of all low molecular weight neurotransmitters and their metabolites. The method is advantageous because it provides a comprehensive view of neurochemical changes that occur with a perturbation or during behavior. The use of LC-MS also allows using stable isotope tracer experiments to track brain metabolism and improve quantification. In this talk we will describe: 1) the method; 2) applications to pharmacology that parallel those done with voltammetry to illustrate the comparison of the methods; 3) novel uses of the method that improve measurement of amino acid neurotransmitters. We will also describe improvements in use of capillary LC-MS that expand its use from opioid peptides to neuropeptides, orexin, vasopressin, and oxytocin. Finally, the sensitivity of the method is compatible with smaller sampling probes. We have developed microfabricated probes that reduce the size of sampling of over 100 fold. This allows better spatial resolution for brain activity mapping.

Keywords: Biological Samples, Chromatography, Liquid Chromatography/Mass Spectroscopy, Metabolomics, Methodology Code: Neurochemistry
Methodology Code: Liquid Chromatography/Mass Spectrometry
Most behavioural studies using microdialysis sampling require tethering of the animal to the microdialysis system so that the animal is freely moving but not freely roaming. In this paper, we describe an on-animal separation-based sensor that combines microdialysis sampling with microchip electrophoresis. The goal is to develop a miniaturized device that can be placed on-animal and is capable of continuous monitoring of drug and neurotransmitter concentrations in the brains of freely roaming sheep. Such a device, combined with video recording, will make it possible to directly correlate neurochemistry with animal behaviour. Microchip electrophoresis is employed for the analysis since it makes possible the separation and detection of several analytes simultaneously with good temporal resolution. Analytes are detected using electrochemical detection, a mode particularly well-suited to such portable analysis systems since the electrode and the potentiostat are easily miniaturized. The current on-animal system is about the size of a lunch box and is run by a laptop battery. The instrument is remotely controlled using telemetry. This system was first demonstrated by monitoring the generation of nitric oxide from subcutaneous infusions of nitroglycerin in freely roaming sheep. Recent progress in the development of an on-animal sensor for the continuous monitoring of biogenic amines in brain microdialysis samples will be presented.

Keywords: Electrochemistry, Electrophoresis, Lab-on-a-Chip/Microfluidics, Sampling
Application Code: Neurochemistry
Methodology Code: Microfluidics/Lab-on-a-Chip
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.

Keywords: Bioanalytical, Electrochemistry, Electrodes, Mass Spectrometry
Application Code: Bioanalytical
Methodology Code: Mass Spectrometry
Depression, a neurological disease, debilitates the lives of millions of Americans every year. Anti-depressants are some of the most commonly prescribed medications in the USA, however these medications are variable in therapeutic outcome and have low efficacy rates. Because the in-vivo neurochemical mechanisms of anti-depressants are poorly understood, it is difficult to design them to be more therapeutically effective. Moreover, anti-depressant screening is primarily based on behavioral tests in rodents and no significant correlations have been reached between these tests and clinical outcome. The most common anti-depressants, selective-serotonin reuptake inhibitors (SSRIs), target the brain’s serotonin system. Fast scan cyclic voltammetry (FSCV) at carbon fiber microelectrodes is a powerful tool for studying in vivo serotonin dynamics. With FSCV, we have previously identified important in vivo serotoninergic mechanisms. We also assessed the effects of systemic administration of SSRIs and found that after a single dose of SSRI, the serotonin signal was changeable within 2 hours, indicating short-term synaptic regulatory mechanisms. In this work, we find that serotonin measurements over 2 hours following SSRI administration have different profiles unique to each different anti-depressant. We create 3-dimensional mathematical models, based on Michaelis-Menten Kinetics, that describe each antidepressant profile. By training the model, we show that it can be used to positively correlate antidepressant efficacy to clinical outcome. We hence present the first voltammetric neurochemical screening method for antidepressant efficacy.

Keywords: Bioanalytical, Micro electrode, Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
The new technique we recently developed (1, 2) (Targeted Plasmonically Enhanced Single Cell Imaging Spectroscopy) has been used to follow the mechanisms of death of single cancer cells exposed to cancer drugs or induced by necrosis-causing agents as well as the dynamics of drug delivery (3).

3) Kang, Bin , Afifi, Mawa and El-Sayed, Mostafa ; Exploiting the Nanoparticle Plasmon Effect: Observing Drug Delivery Dynamics in Single Cells via Raman/Fluorescence Imaging Spectroscopy; ACS Nano, on line.
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 and 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 imaging, surgical delineation 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.

Keywords: Medical, Nanotechnology
Application Code: Other (Specify)
Methodology Code: Other (Specify)
Developing Nanoscale Measurements for the Brain

Since important functions of the brain occur at the nanoscale, it is anticipated that nanoscale tools can be developed to study and to interact with the brain and its component parts (see Alivisatos et al., ACS Nano, 2013). We and others have moved to develop these tools and methods. In our initial work, we functionalized surfaces with isolated and tethered neurotransmitters. These capture surfaces are being used to pull down the membrane-associated proteins from the brain involved in neurotransmission as well as to select molecules to use as artificial receptors for in vivo measurements. The latter will be used to study chemical neurotransmission dynamically at high spatial resolution in many simultaneous parallel measurements. Parallel measurements of voltage activity are further along, such that several thousand measurements can now be made simultaneously with a single multiplexed probe. Ultimately, the great heterogeneity of the brain will require many parallel and specific chemical and voltage measurements, so as to understand and to stimulate neural circuits. This understanding is the goal of the recently announced Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Initiative in the United States. With an initial focus on technology development, there are great opportunities not only to study the brain, but also to understand to learn to treat diseases of the brain. Understanding how neural circuits function and how they function will be critical to these efforts.

Keywords: Nanotechnology, Neurochemistry, Sensors
Application Code: Neurochemistry
Methodology Code: Sensors
It has been generally recognized that chromophore aggregation quenches fluorescence process. We have observed an opposite phenomenon termed “aggregation-induced emission” (AIE) and identified the restriction of intramolecular rotation (RIR) as the main cause for the AIE effect. Guided by the RIR mechanism, we have developed a wide variety of new AIE fluorogens with emission colors covering the whole visible spectrum, fluorescence quantum yields up to unity, and remarkable susceptibility to multi-photon excitation. Nanoparticles of the AIE luminogens (alias “AIE dots”) with efficient fluorescence and excellent biocompatibility can be readily fabricated. In this talk, I will demonstrate the utility of the AIE dots as sensitive and selective probes for in vitro and in vivo biomedical applications.

Keywords: Bioanalytical, Biomedical, Biosensors

Application Code: Bioanalytical

Methodology Code: Fluorescence/Luminescence
For biochemical analysis and biochemical device fabrication, patterning and modifying a solid surface by functional molecules, and making biomolecules in a regular dispersion on a solid surface are essentially important issues, which could be realized by nanoscience and nanotechnology, especially by surface molecular engineering. As a bottom-up technique, self-assembly is considered to be a powerful method in surface molecular engineering. The precise control of nanostructure and arrangement of molecule is now becoming possible in a molecular assembly. In this presentation, the methodology for preparing molecular nanostructures on different substrates such as Au(111), Cu(111) and HOPG are reported. The main methods include non-induced formation and induced deposition. In induced deposition, external stimulations of thermal/electrode potential/ light irradiation, molecular template and local chemical condition are well employed. The assembly with the individual molecules could also be organized and polymerized into molecular nanowires, nanonetworks and various patterns. The structures are investigated by scanning tunneling microscopy (STM) in ambient and electrolyte solution. On the other hand, biomolecules can be also deposited on solid surface. For instance, amino acid molecules formed a (4 x 4) monolayer on Cu(111) surface. With the so-prepared molecular nanopatterns, biomolecules such as peptides can be deposited in the cavity of the network and mono-dispersed on solid surface, forming well-defined molecular nanostructure with different symmetry and molecular ratio. The patterned and modified surfaces are also sensitive to special chemicals, showing potentials in biomolecular analysis and biochemical sensor fabrication.

Keywords: Bioanalytical, Biosensors, Biotechnology, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Sensors
Light microscopy has long been the method of choice to study dynamic processes at the cellular level. However, the resolution of light microscopy is well known to be limited by the diffraction of light, leaving most subcellular structures unresolved. The recently developed super-resolution microscopy techniques lifted this limitation, and pushed the resolution to nearly macromolecular level. We developed super-resolution microscopy techniques for tissue imaging and used a combination of Stochastic Optical Reconstruction Microscopy (STORM) and Structured Illumination Microscopy (SIM) to uncover the molecular mechanism that controls the dendrite growth site in Drosophila embryo motoneurons.
Array tomography is a high-resolution proteomic imaging method that allows the conjugate imaging of both the molecular content and ultrastructure of tissues. This method is based on physical ultrathin serial sectioning, immunostaining and acquiring fluorescence and electron microscopy images of resin embedded tissues, followed by computational volume reconstruction and analysis. Using fluorescence microscopy (FM), dozens of different antibodies and other fluorescent markers can be imaged at synaptic resolution within large volumes of tissue. The same arrays can also be viewed using field emission scanning electron microscopy (FESEM), which allows for the classic benefits of electron microscopy, such as observation of the tissue ultrastructure and tracing of neural wiring and synaptic contacts. By enabling conjugate proteomic and ultrastructural analysis, array tomography data can reveal a previously unseen level of subcellular molecular organization.

Two examples will be presented: one, demonstrating the wide range of molecular diversity of cortical synapses, and the second, revealing the unexpected organization of myelinated axons in cortical grey matter. In both of these cases, the conjugate proteomic and ultrastructural analysis is critically important for the validation, analysis and interpretation of imaging data. Such integration of different imaging modalities can substantially enrich our understanding of the brain, for example by providing molecular context to neural circuit mapping.
I will describe an automated method, which we call serial two-photon (STP) tomography, that achieves high-throughput fluorescence imaging of whole mouse brains by integrating two-photon microscopy and tissue sectioning. STP tomography generates high-resolution datasets that are free of distortions and can be readily warped in 3D for anatomical registration to mouse brain atlases and statistical comparisons between datasets in voxelized co-registered brain volumes. We have established two main applications of STP tomography to the study of mouse brain circuits. In the first application, we use STP tomography for imaging transgenic “indicator” mouse lines expressing GFP from the promoters of activity-regulated immediate early genes (IEGs), such as c-fos and Arc. Computational analysis of whole-brain c-fos-GFP and Arc-GFP datasets allows unbiased mapping of IEG expression, and by extension neural activation, in the mouse brain induced, for example, during behavioral tasks or in response to sensory stimulation. In the second application, we use STP tomography for imaging mouse brains injected with fluorescent anterograde and retrograde tracers, which allows standardized whole-brain anatomical tracing. In summary, STP tomography opens the door to routine systematic studies of mouse brain circuits, both under normal conditions and in mouse models of human brain disorders.

Keywords: Bioinformatics, Fluorescence, Neural Network
Application Code: Biomedical
Methodology Code: Fluorescence/Luminescence
Metal and semiconductor nanoparticles in the 1-10 nm size range are of considerable current interest, not only because of their unique size-dependent properties but also their dimensional similarities with biological macromolecules (e.g., nucleic acids and proteins). These similarities could allow an integration of nanotechnology and biology, leading to major advances in medical diagnostics, prognostics, and targeted therapeutics. In this talk, I present recent development of multifunctional nanoparticles for high-resolution proteomic imaging in cells and tissues.

**Keywords:** Biotechnology, Fluorescence, Imaging, Nanotechnology

**Application Code:** Nanotechnology

**Methodology Code:** Fluorescence/Luminescence
Proteomic Imaging of Ultrastructure Brain Tissue

Fluorogenic Detection of Proteins, Nucleic Acids and Small Metabolites for Cell and Tissue Imaging

Detection of molecules in complex specimens typically involves extensive processing. We have developed a range of fluorogenic detection approaches that can be applied in living and fixed tissues for homogeneous detection of DNA, RNA, Proteins, small molecules and ions. Utilizing fluorogenic dyes and context-specific activation of fluorescence, we are able to efficiently label structures and activities in complex environments. Applications of this technology to functional and molecular imaging of neural cells and tissues will be illustrated.

Co-Authors

Keywords: Biosensors, Detection, Imaging, Microscopy
Application Code: Neurochemistry
Methodology Code: Fluorescence/Luminescence
Supercritical fluid chromatography (SFC) has been a well established technique in drug discovery. However, most of SFC applications have been in chiral resolution – being in the analytical or preparative scales. With the latest development in instrumentation, expansion in column selections, and advance in fundamental mechanistic understandings, SFC has increasingly been applied beyond chiral separations. We present herein recent examples of using SFC to solve critical program challenges. The recent study of pressure effects on SFC separations, and investigation of novel SFC method development strategy will also be discussed, focusing on optimizing tandem column SFC for the separation of complex mixtures.

Keywords: Chiral, Chromatography, SFC
Application Code: Pharmaceutical
Methodology Code: Separation Sciences
## Session # 2140  Abstract # 2140-2  Symposia

**Session Title**: Toward a Preferred Instrument for Gram Scale Supercritical Fluid Chromatography (SFC) Purification

**Abstract Title**: Recent Progress in the Development of Gram Scale Preparative SFC Instrumentation

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### Co-Author(s)

**Abstract Text**

Preparative SFC has been widely used in gram to kilogram scale purifications of intermediates, active ingredients, and other compounds in pharmaceutical industry, primarily in the chiral realm. Recent years have witnessed the expansion of prep SFC applications in areas such as natural products, agrochemical and chemical material. As a result, there has been an increasing need for instrumentation advancement to support a more efficient and cost-effective purification effort. In this presentation, the evolution of the preparative SFC in fundamental principles, instrument design, and practices will be reviewed. In particular, the hardware advancement to accommodate a more user-friendly, more versatile, and more robust prep SFC instrument will be discussed.

**Keywords**: Chiral Separations, Chromatography, Separation Sciences, SFC

**Application Code**: General Interest

**Methodology Code**: Separation Sciences
In this study we evaluated the performance of the JASCO Preparative SFC system for use in gram scale purifications. The two most important parameters for a preparative system, purity and recovery, were tested throughout the dynamic range of the system. Versatility is an important aspect of a gram scale system as injections can vary from very large to quite small and compounds can vary as far as detectability. The injection and detection options were assessed for reproducibility and applicability for various types of purifications, both chiral and achiral. Lastly, as experience from user to user varies, ease of use of any system is very important and the user friendly aspects of the system are discussed and evaluated.
The application area covered by the designation of gram scale separations in SFC can include a range of separations from the high-throughput isolation of an undefined number of major components from a large number of relatively unknown mixtures to enantioseparations involving pure, well-defined racemic or diastereomeric compounds. The needs of the users are simple: reliable systems which give high production rates, product purity and recovery with intuitive operating software consistent through the range of equipment and increasingly Additionally there is an increasing need to minimize the cost and environmental impact of the operation. A range of chromatographic systems has been developed with a commonality of hardware design to ensure simple and reliable scale-up of separations and with a common software platform which retains sufficient flexibility to accommodate system modifications while presenting a consistent user interface.

This presentation describes the challenges - and their solutions - arising from the extension of an existing range of preparative SFC equipment to encompass new systems allowing both open-bed and more conventional, larger-scale separations while using a common software and hardware platform which allows both CO\textsubscript{2} recycling and an alternative injection technique.
### Abstract Text

As gram scale SFC purification becomes more routinely utilized and widely adopted across industry and academia, the need for simple and reliable, high performance instrumentation is ever-increasing. In this presentation we take stock of the current state of the technology, reviewing current options in the marketplace, and looking forward to next generation gram scale SFC tools that will make the utilization of this technology more easily adopted by a growing number of users in new areas. In particular, we discuss the particular needs for academia, and for contract separation organizations, both important sectors in the new prep SFC economy. Finally, we open the floor for general discussion among attendees and all presenters on emerging trend and future directions in the gram scale prep SFC area.

**Keywords:** Chiral Separations, Prep Chromatography, Separation Sciences, SFC

**Application Code:** Drug Discovery

**Methodology Code:** Separation Sciences
Women comprise almost 50% of the US workforce. It would make sense, then, that they should comprise almost 50% of any one profession. But it is not true. In the sciences, the number of women is much lower than their male counterparts. But it does not start out that way. In many undergraduate chemistry programs, women outnumber men. The ACS reported in a 2011 survey that almost 40% of the new Ph.D.’s in chemistry were awarded to women. So what happens and how have the successful women gotten to where they are? This presentation hopefully provides my motivation for organizing this session and set the stage for the rest of the speakers to describe their experiences.

Keywords: Spectroscopy, Vibrational Spectroscopy
Application Code: Other (Specify)
Methodology Code: Other (Specify)
The symposium of Women in Spectroscopy can address many topics such as general obstacles for women in sciences and what it takes to overcome them or the balancing act of family and careers. As a woman entrepreneur, who co-founded a spectroscopy-based business, both topics are of interest but in this talk we will touch on the latter. Can a woman really have it all? - successful career, children, husband, volunteer for societies and causes and yet find time for herself? There are many successful women in politics and business - is it harder for us in sciences? Is there a magic bullet for 'success' and how does one define 'success'? In this talk, I will share some of my experiences and lessons learned for good vibrations in the lab and at home with hopes to inspire the young women scientists to reach for their goals, hopes and dreams.

Keywords: Biopharmaceutical, Characterization, Surface Enhanced Raman, Vibrational Spectroscopy

Application Code: General Interest

Methodology Code: Vibrational Spectroscopy
This talk will explore career options at primarily undergraduate institutions (PUI). While these institutions do not have graduate students, how much research is valued varies dramatically between them. Teaching expectations are often quite high, not only in terms of teaching load, but in the high expectations for quality and innovation in teaching. Service is often a more important part of a faculty position at these institutions as well because their small faculties are highly engaged institutional governance. Advice will be given on how to present yourself to be competitive for these positions and what to expect during the search process, as well as how to prepare yourself to be successful once hired. Special attention will be paid to the unique challenges analytical chemists face in teaching and performing research at these kinds of institutions.

Keywords: Education
Application Code: General Interest
Methodology Code: Other (Specify)
In this talk I will share my observations about academic careers based on my accumulated observations over 30+ years as a university professor. I will discuss my own wanderings in academics, from building and sustaining a research program to a recent stint as department head. The focus will be on the importance of individuality – forging your own path based on your (likely shifting) priorities and goals, identifying what is most important and keeping it front and center, and recognizing the importance of women as friends and colleagues.
Each of us come to the world with our talents and skills, and sometimes have many options before us on how to use these. It is a personal path that can sometimes be littered with road blocks. There is no silver bullet to how succeed as a woman in spectroscopy – but it can be argued that regardless of gender hard work, planning and serendipity play a role in success.
A career in the field of Spectroscopy offers an opportunity to make a positive impact on the quality of life for people around the world. This speaker's journey has included working as a spectroscopic in a consumer products industry, managing international organizations of scientists and engineers and, more recently, designing program sessions to recognize "Analytical Chemists Easing World Poverty." Each stage in this journey has brought new awareness of:

- The importance of paying attention to scientific details,
- The importance of looking at a challenge from multiple points of view, and
- A responsibility to address the unmet human need for measurement solutions around the world.

This talk will include examples of specific gaps in measurement capability essential to insuring safe drinking water, food and pharmaceuticals, as well as adequate waste management. It will explore how even well-intended organizations, like the World Health Organization, can create unintended challenges for analytical methods and instruments, and how some surprising parallels exist between analytical chemistry and every other industry working in the same region. It will also report on some of the significant activity underway to try to bridge existing world measurement gaps.

Keywords: Environmental/Waste/Sludge, Food Science, Pharmaceutical, Water
Application Code: General Interest
Methodology Code: Other (Specify)
Today, there are many options available to women with Ph.D.s in chemistry. The typical preparation in most Ph.D. programs tends to be in research-oriented professions, such as in Academia, Pharma, Manufacturing, etc. However, circumstances sometimes force new graduates to choose a path that is unrelated to academic research and industrial R&D. In the early stages of a career, it is best to be open-minded, because it can lead graduates to careers that are both intellectually satisfying and financially rewarding. In my own experience, I was seeking a path that would enable me to remain in the US and work in a position that was challenging, technically. To that end, my professional history in spectroscopy started with an applications chemist position in atomic spectroscopy for a major instrument manufacturer. While in that position, I was recruited – because of my Ph.D. research - by a major chromatographic instrument manufacturer, to another applications chemist role, a job which provided a significant financial improvement over my previous job and eventually provided me with the incentive to think about other options. After working in that position for several years, I started to feel stifled by being restricted to laboratory work and was, fortunately, offered a position in technical sales of molecular spectroscopy products, which I accepted. Because I took a chance by working in a non-research oriented job at the beginning of my career, I eventually recognized that my preference is to work outside of the laboratory, providing advice on spectroscopy products as a function of my consultative sales position. I believe that women now have many options open to them, including academic research positions to upper management functions. We should not be afraid to take a chance on that first job, even if it isn’t research-oriented, because it can lead to a job that ultimately is the best fit both personally and financially.

Keywords: Spectroscopy
Application Code: General Interest
Methodology Code: Molecular Spectroscopy
Advances in Catalysis and Hydrocarbon Analysis

Trace Analysis of Total Sulfur and Nitrogen in Hydrocarbon Matrixes by Combustion and UV Fluorescence and Chemiluminescence: Optimization of Analytical Parameters

Combustion of hydrocarbons followed by UV-Fluorescence and Ozone Chemiluminescence has become a preferred method to determine trace level amounts of sulfur and nitrogen respectively in gases, liquids and solid samples. This analytical technique is very selective, equimolar in instrumental response and exhibits a wide linear dynamic range. A Limit of Detection of 15 and 10 g/Kg, for S and N respectively has been determined by liquid direct injection for the new instrument configuration considered in this work. Several important applications for the simultaneous determination of total sulfur and nitrogen in Reformulated Gasoline, hydrodesulfurized naphthas, reformates and diesels matrixes are discussed in detail. The Simultaneous determination is performed on the hydrocarbon combustion gases utilizing chemiluminescence detection for nitrogen and UV-Fluorescence for sulfur. Important considerations regarding the hardware and the statistical analysis of the experimental data are discussed. Results show that stability of the analytical system, linearity of detectors and high repeatability are key parameters to achieve low limits of detection. Comparison to ASTM Inter Laboratory Proficiency Programs shows excellent agreement. Matrix effects if any can easily be minimized by standard addition and results correlate with standard methods at normal and reduced pressures. This procedure is fast, easy, very specific and sensitive.

Keywords: Elemental Analysis, Fluorescence, Fuels\Energy\Petrochemical, Sulfur
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Carbon dioxide (CO$_2$) is a readily available industrial byproduct that can be used to produce useful chemical compounds. Electrochemical reduction is a highly effective method for converting CO$_2$ to CO, organic acids, low molecular weight hydrocarbons and alcohols. Product distribution from the reduction of CO$_2$ is highly dependent on the composition, preparation of the catalysts and reduction potential. Copper-based catalysts have been found to produce higher molecular weight hydrocarbons, such as ethylene, methanol and ethanol. This project focused on the production of monolayer copper catalysts, producing lattice parameter mismatch, resulting in novel catalytic properties.

A custom build acrylic flow cell was used to investigate the products of CO$_2$ reduction on various copper-based catalysts with a Ag/AgCl (3 M NaCl) reference electrode, a Nafion® divider (Alfa Aesar 0.05mm), and a DSA® counter electrode (De Nora). All electrochemistry was performed on a PAR 236A potentiostat and 1260 Solartron FRA. All catalysts were analyzed in a pre-electrolyzed KHCO$_3$ (0.1 M) solution saturated with CO$_2$ at 1 atm. The headspace gas was analyzed in real time by flowing the head space gasses (5 mL/min) to a GC (7890A). The gaseous products were detected via mass spectrometry (9475C) as well as thermal conductivity. Aliquots of the electrolyte were collected to be analyzed via Ion chromatography (IC, Dionex 1500) and NMR (Bruker DPS). Preliminary results show that copper monolayers on a Au (111) substrate have a product distribution uniquely different from pure gold or copper.
Petroleum is the most complex matrix in nature, constituted by many thousands of compounds. Petroleum analysis presents an analytical challenge, and for mass spectrometry analysis we need equipment with high resolving power and high mass accuracy. Because of that, Fourier transform ion cyclotron resonance mass spectrometer has been the gold standard for comprehensive analysis of crude oil and petroleomic studies. The complex nature of petroleum demands analytical solutions and instrumental methods that will identify components in the complex chemical profile as well as characterize key functional group class differences. Advances in the resolution and mass accuracy in time-of-flight mass spectrometry provides an alternative for most routine petroleomic studies. In this study we compare the composition of crude oil samples from different origins through high resolution time-of-flight mass spectrometry platforms. The complementary information obtained by gas chromatography techniques (one dimension and comprehensive two dimensional) for analysis of volatile and semi volatile compounds of crude oils and electrospray ionization mass spectrometry of polar compounds allow the full characterization and classification of petroleum samples.

Keywords: Electrospray, GC-MS, Petroleum, Time of Flight MS
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Mass Spectrometry
We describe the design and synthesis of Pt-Cu electrocatalysts with well-defined, tunable morphology and composition. Electron microscopy and Brunauer-Emmett-Teller (BET) surface analysis indicate our catalysts have extremely high porosity, which acts to maximize mass transport, increase active surface area, and minimize the overall precious metal content. Production of these catalysts is quite facile and begins with synthesis of a porous Cu substrate, formed by etching Al from a Cu-Al alloy. The porous Cu substrate is then coated with a Pt layer via galvanic replacement with K2PtCl4. Growth of the Pt layer is manipulated by time, temperature, concentration of K2PtCl4, and convection rate during galvanic replacement. Data from inductively coupled plasma-mass spectrometry (ICP-MS) and energy dispersive spectroscopy (EDS) confirm multiple Pt loadings have been achieved via the galvanic replacement process. The Pt layer was found to produce a fivefold enhancement in oxygen reduction activity relative to the current state-of-the-art Pt/C catalyst and act as a barrier towards corrosion of the Cu understructure. The high ORR activity obtained indicates that high catalytic activity could potentially be achieved from fine-tuning this technique for other bimetallic Pt-based catalysts.

Keywords: Electrochemistry, Energy, Fuels\Energy\Petrochemical, Voltammetry
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Electrochemistry
Advances in Catalysis and Hydrocarbon Analysis

Fuel Quality Verification in 30 Seconds at the Point of Receipt Using a Military Grade Raman Spectrometer

In many countries, covert fuel theft is executed by diluted shipments with less expensive petroleum products. Not surprisingly, this leads to decreased engine performance and ultimately engine failure. Fuel quality is defined in terms of its chemical and physical properties that dictate performance. These properties include aromatic, olefinic, naphthene, oxygenate, saturate and sulfur content, cetane number, cloud point, density (API gravity), distillation points, flash point, freezing point, heat of combustion, octane number, pour point, Reid vapor pressure, thermal stability, and viscosity. Although most of the chemical properties can be determined by a gas chromatograph within 30 minutes, many of the physical properties each require a unique piece of apparatus and 30 to 60 minutes to perform. In general, a complete characterization of a fuel sample takes from 6 to 8 hours. Consequently, there exists a need to rapidly verify fuel quality upon receipt. Often, such verification must be performed far from a lab containing standard fuel analysis instruments and apparatus. To satisfy this need, we have developed a 35 lb, brief case-sized, battery-operated, fuel analyzer that can survive transport vibrations and shock, operate in blowing sand and rain from -4 to 110 °F. The analysis employs FT-Raman spectroscopy and chemometrics to determine the 18 properties listed above within 30 seconds, using only 20 mL of sample. This presentation describes the analyzer and the chemometric analysis.

Keywords: Chemometrics, Fuels\Energy\Petrochemical, Petroleum, Raman
Application Code: Fuels, Energy and Petrochemical
Methodology Code: Vibrational Spectroscopy
To meet current and future emission standards, diesel engines are often outfitted with filters for particulate matter emission reduction. To achieve a filter regeneration during city- and low-temperature-cycles, the temperature of the filter element can be increased by a late fuel post-injection into the cylinder. As a side effect during this process, significant quantities of the injected and unburned fuel deposit on the cylinder wall, mix with the engine oil and subsequently get washed down into the oil sump.

To optimize the injection and minimize the fuel ingress into the engine oil, it is important to examine the quantity and chemical constitution of the diesel fuel entering the crankcase as well as the fuel leaving the crankcase through the ventilation system.

With these objectives a system consisting of a heated multiport inlet system, a gas chromatograph and a quadrupole mass spectrometer was built and tested. During measurements it is placed directly next to the engine in a test cell.

Via the multiport valve, an aerosol sample chosen from one of three distinct positions at the engine can be transferred into a high temperature evaporator, the split injector and following the GC capillary. Retention times and injection quantity can be optimized for the application through the variation of injector vacuum, injection time and GC temperature. Additionally, the system is equipped with an evaporator and two piezo valves for online calibration. The total time between two samples can be as short as 120 seconds.

With this setup, it is possible to effectively monitor the whole process of fuel ingress into the lubricant.

**Keywords:** Aerosols/Particulates, Gas Chromatography/Mass Spectrometry, On-line, Sample Handling/Automation

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

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Comprehensive two dimensional GCxGC-MS significantly improves GC separation. However, it suffers from complexity and high cost of purchase and cooling gases. In addition, with standard electron ionization the molecular ions are often weak or absent particularly in complex hydrocarbon mixtures analysis. We developed a pulsed flow modulation (PFM) technique for obtaining GCxGC-MS with supersonic molecular beams (SMB) (also named Cold EI) based on quadrupole mass analyzer and without any added gas. Sample compounds which elute from the first GC column are temporarily stored in a fused silica transfer line and are pulsed periodically injected by ~25 ml/min He gas pulse into the second column. After the pulse, 20 ml/min He develops the chromatography in the second column for a few seconds. PFM is simple to construct, does not require any added gas and the injection time can be tuned, hence GCxGC-MS with Cold EI is enabled with quadrupole MS, and the increased PFM flow rate is handled by the nozzle chamber differential pumping. We combined PFM-GCxGC with the 5975-SMB GC-MS with Cold EI. PFM-GCxGC-MS with Cold EI combines the improved separation of GCxGC with Cold EI features such as enhanced molecular ions and mass spectral isomer and isotope information for the provision of ultimate sample information. Unique to PFM-GCxGC-MS with Cold EI is that as the second dimension elution time is increased the molecular ion mass is reduced for easier GCxGCxMS like identification. Examples with a few samples including JP8 jet fuel will be shown.

**Keywords:** Fuels\Energy\Petrochemical, Gas Chromatography/Mass Spectrometry, GC-MS, Hydrocarbons

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

**Methodology Code:** Gas Chromatography/Mass Spectrometry
Various organic-based monoliths were prepared and optimized for immobilization of the protein human serum albumin (HSA) as a binding agent for chiral separations and high-performance affinity chromatography. These monoliths contained co-polymers based on glycidyl methacrylate (GMA) and ethylene glycol dimethacrylate (EDMA) or GMA and trimethylolpropane trimethacrylate (TRIM). A mixture of cyclohexanol and 1-dodecanol was used as the porogen, with the ratio of these solvents being varied along with the polymerization temperature to generate a library of monoliths. An additional study was also performed to evaluate the effects of novel polymerization modes involving the use of an accelerator, heat and/or light. These monoliths were used with both the Schiff base and epoxy immobilization methods and measured for their final content of HSA. Monoliths showing the highest protein content were further evaluated in chromatographic studies using R/S-warfarin and D/L-tryptophan as model chiral solutes. A 2.6–2.7-fold increase in HSA content was obtained in the final monoliths when compared to similar HSA monoliths prepared according to the literature when the polymerization conditions were only performed using heat. The increased protein content made it possible for the new monoliths to provide higher retention and/or two-fold faster separations for the tested solutes when using 4.6 mm i.d. x 50 mm columns. These monoliths were also used to create 4.6 mm i.d. x 10 mm HSA microcolumns that could separate the same chiral solutes in only 1.5–6.0 min. The approaches used in this study could be extended to the separation of other chiral solutes and to the optimization of organic monoliths for use with additional proteins as binding agents.

Keywords: Bioanalytical, Biopharmaceutical, Chiral Separations, HPLC Columns
Application Code: Bioanalytical
Methodology Code: Liquid Chromatography
Lignin is a highly complex amorphous polymer found in every vascular plant, and the second most abundant renewable carbon source on the planet. Unfortunately, due to its inherently amorphous structure it is considered to be an unusable waste product or a cheap heating fuel. A major challenge in lignin utilization is adequate chemical separation and characterization. Capillary-channeled polymer (C-CP) fibers are being investigated as a stationary phase utilized for the separation of lignins. The fibers have eight micron-sized channels that run collinearly down the length of the fiber inducing a wicking effect via capillary action. C-CP fibers useful for bio macromolecule separations due to: low cost, high surface area, being non-porous, pH stability, and requiring >50% lower backpressures when compared to standard packed bed columns.

Loading was accomplished under 100% MilliQ water, and lignin was eluted with the introduction of organic solvent (ACN and MeOH) at 4mL/min. A thirty minute gradient from 100% MilliQ water to 100% ACN was employed for effective separations. Comparison of the separation between digested and undigested samples showed a decrease in unretained species (nutrients and salts), and a shift in the peak areas of a few select peaks indicating a change in the lignin composition.

Further inquiries will be performed to improve the separation by optimizing column and gradient parameters. Further testing is being completed on additional stationary phases, (poly(ethylene terephthalate) and nylon-6), and mobile phases. After adequate separation is achieved, samples are fractioned and analyzed by MALDI-MS to gain further insight on both bacterial degradation and chromatographic performance.
Separation of enantiomers of a chiral compound is one of the most interesting and challenging tasks because of their identical physical and chemical properties. The magnetic materials possessing chiral functionality on their surface can afford not only magnetic property but also to recognize chirality. The Fe3O4@ZrO2 core-shell nano-materials have not been reported previously for chiral separation of racemates. Zirconia has been shown to be most stable oxide mechanically, thermally and chemically, and shows better physical and chemical properties among inorganic metal oxides. The present work explores core-shell microspheres consisting of a Fe3O4 magnetic core covered by a zirconia shell (Fe3O4@ZrO2) which are immobilized with cellulose tris(3,5-dimethylphenylcarbamate) to get chiral zirconia magnetic microspheres (CZMMs). As-synthesized CZMMs have been applied for the separation of racemic chiral drugs and the results indicate the CZMMs are the potential chiral nanomaterials for enantioseparations. Most importantly the synthesized CZMMs have shown an excellent recyclability and can be used for further chiral separations of different kinds of racemates. The results are interesting and encouraging for further modifications so as to develop more synthetic methods for chiral magnetic materials.

Keywords: Chiral Separations, Drugs, HPLC, Materials Characterization

Application Code: Bioanalytical

Methodology Code: Liquid Chromatography
Bioanalytical Separations

Measurement of the Secretion Dynamics of Islets of Langerhans Using a Microfluidic Device

Recent development of novel, online monitoring systems has opened up a myriad of opportunities for the study of cellular dynamics. For example, individual islets of Langerhans secrete insulin in 5-min pulses upon stimulation with a constant glucose concentration resulting in an elevated, but stable, insulin output from the pancreas. Yet, it is believed that if these islets are stimulated by an oscillatory glucose concentration with the appropriate frequency, the islets will synchronize and secrete insulin in unison resulting in a pulsatile insulin output. This study aims to test this theory by delivering a glucose waveform to stimulate the secretions of batches of islets of Langerhans. This novel phenomena and method, in turn, aims to elucidate the non-linear dynamics of islets of Langerhans.

The analytical device to perform these measurements consists of a two-layer microfluidic glass chip containing both a perfusion system for delivery of the glucose waveforms and a separation channel for measurement of insulin secretion by an electrophoretic competitive immunoassay. Islet secretions were mixed online with Cy5-labeled insulin and anti-insulin antibodies that were sampled and separated by electroosmosis at 2 nL/min. The setup yielded a 13-s sampling frequency, and the ability to quantify 100-fold concentration differences in islet-secreted insulin. Calibration curves were obtained for 0-1 [micro]M insulin with relative standard deviations <5% for all points and a limit of detection of 4 nM. Thus, the device provides an automated method for stimulating islets with glucose waveforms and monitoring insulin secretion profiles in real time with high temporal resolution.

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

Application Code: Bioanalytical

Methodology Code: Microfluidics/Lab-on-a-Chip
Bioanalytical Separations

New Advances in Stationary Phases for Glycan Analysis

Glycans play fundamental roles in cellular function by creating a fingerprint tag for the protein they are bound to, which in turn affects cellular activity. Minor changes in glycan structure can have dramatic impact on cell function. Glycans are often key biomarkers for disease states such as cancer. Due to chain branching and post-translational modifications, the structure of glycans is very complex thus their characterization is highly challenging, requiring a mass spectrometer to decipher glycan structures. Hydrophilic interaction (HILIC), ion exchange (IEX) and reversed-phase (RP) columns can be used for glycan separation by HPLC. Because of their high hydrophilicity, glycans are often analyzed on amide HILIC columns which separate glycans mainly by hydrogen bonding, resulting in a size and composition-based separation. However, for accurate characterization of glycans, development of separation columns that provide better resolution and different selectivity are needed. This presentation will discuss the latest developments in stationary phases for glycan separation, with the focus on mixed-mode chromatography and superficially porous particles. It has been found that compared to conventional HILIC method, mixed-mode chromatography provides distinctive selectivity and superior resolution. Examples will be given on native and labeled glycans from bovine fetuin and from Human IgG using LC-fluorescence and LC-MS methods.

Keywords: Bioanalytical, HPLC Columns, Liquid Chromatography/Mass Spectroscopy, Pharmaceutical

Application Code: Bioanalytical

Methodology Code: Liquid Chromatography/Mass Spectrometry
Development of Peptide Reporters for Monitoring E3 Ligase and Proteasome Activity in Single Cells

Dysregulation of the ubiquitin proteasome system (UPS) is suspected to play a key role in a host of diseases, including cancers such as multiple myeloma and neurodegenerative diseases such as Parkinson’s disease. Inhibitors targeting components of the UPS represent a promising new branch of targeted therapies for these diseases; however, there is currently no assay capable of measuring the activity of these proteins in patient samples. Due to the small sample sizes and presence of mixed cell types, patient samples present unique obstacles to conventional analysis. Faced with these challenges, single-cell analysis provides an ideal strategy for analyzing patient samples.

Here we present a new bioanalytical tool capable of measuring E3 ligase and proteasome activity in biological samples. We developed fluorescent peptide reporters containing degradation signals which are recognized by E3 ligases and ubiquitinated. Ubiquitinated reporters are targeted to the proteasome unless a proteasome inhibitor is present. Capillary electrophoresis with laser induced fluorescence detection (CE-LIF) enables separation and quantification of modified and unmodified reporters to infer enzyme activity. We show results from cell lysate studies using several peptide reporters incorporating various degradation signals including those from p53 and β-catenin. We also report our efforts to adapt reporters to analysis by CE-LIF, as well as work optimizing reporters to enhance ubiquitination rates and increase longevity of the reporters in the cytosolic environment. In the future, combining these reporters with single cell CE-LIF will permit quantification of E3 and proteasome activity in single cells.

Funding is provided by NIH (EB011763, F32CA162574)

Keywords: Bioanalytical, Capillary Electrophoresis, Enzyme Assays, Peptides

Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
**Session Title**: Capillary Electrophoresis: Small Molecules and Neurotransmitters  
**Abstract Title**: Metabolomic and Peptidomic Profiling of Crustacean Neuroendocrine Tissues by Capillary Electrophoresis-electrospray Ionization-Mass Spectrometry  
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**Abstract Text**

The blue crab *Callinectes sapidus* has been used as a model organism for the study of neurobiology and various physiological processes. Here, we employ a new analytical platform that integrates capillary electrophoresis and electrospray ionization mass spectrometry (CE-ESI-MS) for simultaneous profiling of small molecule neurotransmitters and their metabolites, and neuropeptides extracted from the neuroendocrine tissues of *C. sapidus*. A homemade ‘junction-at-the-tip’ type CE-ESI interface was used for online coupling of CE and Waters Synapt G2 Q-ToF mass spectrometer. The capillary column terminus is surrounded by a tapered stainless steel hollow needle, and the interior of the needle tip acted as the CE outlet while its exterior tip surface provides the electrode surface for electrospray ionization. Acidified methanol was used to extract cationic metabolites and neuropeptides from tissues, and the crude extracts were cleaned up by strong cation exchange (SCX) ziptip before CE-ESI-MS analysis. More than 200 molecular species were found in pericardial organ (PO) extract from a single CE-MS run, including approximately 100 singly charged cationic small molecules and 100 multiply charged peptides. Important small molecule neurotransmitters, such as acetylcholine, glutamate, [gamma]-aminobutyric acid, aspartate, as well as several families of neuropeptides, such as allatostatin A-type, RFamide, Orcokinin, were detected in a single CE-MS run. Combining SCX sample preparation with CE-MS also allowed detection of at least 30 peptides that were not observed by C18 nanoLC-MS in previous study. This approach facilitates simultaneous monitoring the changes of small molecule neurotransmitters and neuropeptides under different biological conditions.

**Keywords**: Bioanalytical, Biological Samples, Capillary Electrophoresis  
**Application Code**: Neurochemistry  
**Methodology Code**: Capillary Electrophoresis
Measurement of in vivo neurotransmitters in extracellular spaces can serve as an indicator for brain activities, which is essential for the understanding of neurological and psychological diseases. However, these neurotransmitters at low levels mix with numerous other chemicals thus imposing challenges in resolving them from interferences. An integrated microfluidic system coupled with electrophoretic separation is a powerful tool for dynamically monitoring species of interest, especially in complex biological samples. Here integrated capillary electrophoresis (CE) systems coupled with microdialysis were developed and applied to in vivo neurotransmitter monitoring. The systems were interconnected with prototyped PDMS interfaces with robust performance of long-term continuous injections and separations. Online derivatization of amino acid neurotransmitters was performed with NDA at the presence of cyanide. Conditions were optimized to achieve rapid separations with high efficiency. In addition, powered with microfabricated pneumatic pumps, push-pull microdialysis or perfusion was performed at ultra-slow flow rates which lead to improved sampling recovery thus enhancing detectability based on laser-induced fluorescence (LIF) detection. Furthermore, multiple probes were integrated to monitor various brain locations simultaneously, which provided a strategy for the investigation of brain functional connectivity in terms of specific neurotransmitters. In this talk, related technologies and applications in in vivo monitoring will be presented and discussed.

Keywords: Capillary Electrophoresis, Lab-on-a-Chip/Microfluidics, Neurochemistry, Sampling
Application Code: Neurochemistry
Methodology Code: Capillary Electrophoresis
Sialic acids are a family of 9-carbon carboxylated sugars found at the distal termini of glycoconjugates. About 50 different molecular species of sialic acids are known to occur in nature and the most common is N-acetyl-neuraminic acid (Neu5Ac). They are directly involved in many biological processes. There is continuous interest in developing highly sensitive, selective, and reliable strategies for the determination of sialic acids. We are investigating the use of capillary electrophoresis (CE) with laser-induced fluorescence (LIF) for the determination of sialic acids. One of our goals is to establish capillary electrophoretic profiles of sialic acids from different submaxillary mucins. We have specifically labeled sialic acids with 1,2-diamino-4,5-methylenedioxy-benzene dihydrochloride (DMB) to yield a highly fluorescent quinoxaline derivative that is easily excited with a 375 nm diode laser, allowing for LIF detection. Separation of different sialic acids is accomplished via CE. The effect of several parameters (e.g., pH, organic modifiers, and others) on the CE separation and LIF detection were investigated while using Neu5Ac as a standard sialic acid probe. Sialic acids released from bovine submaxillary mucins by treatment with Clostridium perfringens and Arthrobacter ureafacines were examined by our CE-LIF method. Our presentation will provide details on the different parameters studied to establish the CE-LIF method and our initial analysis of sialic acids from bovine submaxillary mucins.
Sialic acid content is critical to extend the in vivo efficacy of therapeutic drugs. Neuraminidase catalytically cleaves sialic acid groups from glycans and is excellent for evaluating the sialic acid content. To achieve this the enzyme performance must be characterized and the enzyme-based assay validated. Capillary electrophoresis is an ideal method to characterize enzymes and oligosaccharides. Capillary methods require nanoliter volumes of enzyme and incubation time is reduced to minutes. Recently capillary electrophoresis separations of complex glycans were enhanced with phospholipids and glycan composition was identified through microscale sequencing. These phospholipid additives effectively separate glycan isomers and are compatible with enzymes. In addition, these preparations have a thermally dependent viscosity and are easily loaded at lower temperatures, but form the viscous pseudo-gel required for effective capillary separations. Previously, enzyme-based capillary electrophoresis sequencing was applied to Trastazumab. That work revealed the feasibility of integrating sialidase in the enzyme assay, but the assay performance was not investigated further. To extend the investigation, the catalytic efficiency of neuraminidase is determined using phospholipid-assisted capillary electrophoresis. The performance of neuraminidase sequencing to quantify sialic acid content is assessed. In addition, the method is utilized to evaluate bond linkage by monitoring the products obtained with select oligosaccharides and neuraminidase with different specificity.

This material is based upon work supported by the National Science Foundation under award number CHE-1212537 and #1003907.

Drosophila melanogaster is an important model organism due to their short generation time, ease of genetic manipulation, and well understood genome. Our lab is interested in understanding neurotransmission in the fruit fly in order to expand its use as a model organism for human neurological diseases. In order to gain better insight into the neurochemistry of D. melanogaster, methods for the sensitive detection and rapid separation of neurotransmitters are required.

In order to monitor tissue content of neurotransmitters in nerve cord and adult brain samples, particularly histamine and carcinine, which are responsible for visual transduction in invertebrates, our lab has developed capillary electrophoresis coupled to fast scan cyclic voltammetry (CE-FSCV). CE-FSCV provides both low limits of detection and rapid separations for a wide range of monoamine neurotransmitters. Samples are electrokinetically injected and separated, with end capillary FSCV detection provided by a carbon fiber microelectrode positioned 20 [micro]m away from the capillary outlet in a flow cell. Using a phosphate buffer system, analytes (histamine, tyramine, serotonin, carcinine, octopamine, and tyramine) were separated in under 10 minutes. Previous work using CE-FSCV did not focus on the separation of carcinine and histamine. CE-FSCV provides sample identification through both migration times and characteristic cyclic voltammograms, and offers excellent sensitivity, with limits of detection in the 10-100 nM range. Capillaries were treated with polymers (PVA, PEI) in order to modify the separation by reducing both electroosmotic flow and interactions between analytes and the capillary wall. These coatings increased the reproducibility of separations. Increased reproducibility, in addition to short separation times provides an improvement over our previous work with CE-FSCV as well as an advantage over HPLC based methods.

Funding generously provided by the NIH, grant number R01MH085159.
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 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. Our ability to detect basal and stimulated release of amines was confirmed by transferring the probe between artificial cerebrospinal fluid (aCSF) and a potassium-spiked (100 mM K+ aCSF) stimulant solution. Dynamic changes in the relative abundance of analytes were monitored with 20 second temporal resolution. Glycine demonstrated the largest percent increase (700%), followed by taurine (185%) and serine (215%). Though the developed platform is currently being used to investigate the role of astrocytes in neuronal activity, it is suitable for a wide range of models. This work has potential to advance the capabilities and physiological relevance of cell-based assays.
The remarkable diversity of the biological landscape provides an interesting opportunity to use recent advances in separation methods to broaden our understanding of the heterogeneity that exists within the tissues and cells of organisms. It seems intuitive that different tissues will exhibit stark heterogeneity in terms of the chemical composition compared to one another, but this chemical diversity even exists between neighboring cells within the same tissue section thus clearly highlighting the need for single cell analysis. Capillary electrophoresis (CE) as the separation mechanism coupled with laser-induced fluorescence (LIF) detection is a particularly powerful combinatory technique for single cell analysis due to its ability to probe both mass and volume limited samples, produce high separation efficiencies, and detect and quantitate low abundance analytes (Lin et al).

This study hyphenates a single-beam optical trap with CE and multichannel LIF detection. Cell localization is achieved with the trap’s utilization of light’s ability to impart momentum on the order of picoNewtons, and then a micromanipulator controlled using a computer allows for the positioning of the capillary inlet near the cell. Cell lysis is achieved within the capillary inlet via hydrodynamic injection. The sample is then separated using 30 kV in a CE separation and detected via LIF. The system enables injections of individual rate pinealocytes and quantification of their endogenous indoles, including serotonin, N-acetyl-serotonin, 5-hydroxyindole-3-acetic acid, tryptophol and others (Cecala et al).

References

Keywords: Capillary Electrophoresis, Fluorescence, Neurochemistry, Other Hyphenated Techniques
Application Code: Neurochemistry
Methodology Code: Capillary Electrophoresis
Capillary electrophoresis (CE) is well known for its high separation efficiency and broad sample compatibility. The utility of CE separation has been well demonstrated in a broad range of chemical and biological applications. The major limitation of CE based separation is its limited sample loading capacity and measurement dynamic range. Transient isotachophoresis overcomes these limitations by allowing large sample loading volume and targeted enrichment of low abundance components in a complex sample mixture. A hybrid capillary isotachophoresis (CITP) and capillary zone electrophoresis (CZE) separation coupled with electrospray ionization mass spectrometry (ESI-MS) can be an idea technique for highly sensitive and quantitative measurement of targeted protein biomarkers in biofluids. The major challenge is the development of a highly efficient interface to couple CITP/CZE with MS. We present the development of a novel sheathless CITP/CZE-MS interface that simultaneously achieves large sample loading capacity and stable nanoflow ESI (nanoESI) operation with no sample dilution. The new interface effectively resolves the challenging mismatch between the need to use large i.d. separation capillary for large sample loading capacity and small i.d. emitter capillary for nanoESI operation in all the existing CE-MS interface designs. Detailed characterization of CITP/CZE-MS using the new interface will be presented to show its achievable sample loading capacity, separation peak capacity and reproducibility. The direct coupling of (CITP/CZE) with a triple quadrupole mass spectrometer operating in a selected reaction monitoring (SRM) mode will also be presented to demonstrate its achievable low limit of quantitation (LOQ) by analyzing targeted peptides in biological matrix. Preliminary experimental data showed a linear dynamic range spanning 4.5 orders of magnitude and a 10 pM LOQ with excellent measurement reproducibility using the new sheathless CITP/CZE-nanoESI-SRM MS.

Keywords: Bioanalytical, Capillary Electrophoresis, Quadrupole MS, Quantitative
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Two dimensional cell culturing is the primary means for investigation of animal tissue cells but is limited by a number of constraints, most notably the limited lifespan of individual cultures and a failure to reproduce the in vivo conditions that cells and tissues would typically experience. Various bioreactors have been designed to simulate more in vivo-like conditions for cell culturing as an answer to these limitations. Strong evidence exists that bioreactor conditions can dramatically affect the metabolic activity of cells, in many cases significantly increasing the yield of many biomolecules on a per cell basis. Awareness of the metabolic behavior of cells is essential both for maintenance of cell cultures and experimental investigations. Current quantitative sampling techniques for bioreactors are predominately spectroscopic or off-line methods. A screen-printed electrode (SPE) array encased in a microfluidic flow chamber has been developed for use in highly specific real time electrochemical monitoring of cellular metabolites including glucose, lactate, oxygen, and extracellular acidification in the effluent of a bioreactor chamber. The SPE is comprised of five platinum electrodes which detect a wide variety of analytes through the application of various selective enzymes and metal films. Individual sensors are inexpensive, reusable, have a lifetime of several weeks, and are easily customized. The capabilities of this method are demonstrated by real-time monitoring of cellular responses to well-studied metabolic stimuli, and validated by comparison to established methods.

Keywords: Chemically Modified Electrodes, Electrochemistry, Instrumentation, Toxicology
Application Code: Biomedical
Methodology Code: Electrochemistry
Carbon-fiber microelectrodes have been extensively investigated for analytical and neuroscience applications because of their excellent chemical and electrochemical stability, remarkable electrical and mechanical properties, unique catalytic activity and good biocompatibility. Recently, carbon-nanotube microelectrodes have gained attention for the purpose owing to their potential to further promote electron-transfer reactions and to tolerate surface fouling. Biochar is the carbon-rich product when biomass (such as wood and crop residues) is heated in a closed container with little or no available air. High surface area, good conductivity, excellent stability, unique fingerprint microstructure and low-cost of the biochar enable it to be a new class of carbon-based electrochemical materials. Therefore, it is interesting to study biochar microelectrodes (BCF) as an alternative to carbon-fiber and carbon-nanotube microelectrodes.

In this work, we have successfully developed a BCF microelectrode which is prepared by simply sealing a BCF into a glass pipette via a conventional heat-sealing approach. Because the BCF can be easily prepared via the pyrolysis or gasification of biomass wastes, there are no cost and availability issues. Because it possesses regular macropores separated by carbon walls, it has the potential to integrate the advantages of carbon-fibers and those of carbon-nanotubes. Because it can be easily sealed into the glass, the BCF microelectrode can withstand mechanical polishing and cleaning. We have demonstrated that the BCF microelectrode retains the merits of a regular microelectrode. We have studied the reduction and oxidation of nitrite on the microelectrode as an example of its potential sensing applications.

Keywords: Electrochemistry, Environmental Analysis, Microelectrode, Sensors
Application Code: Environmental
Methodology Code: Electrochemistry
Amperometric detection using microelectrodes integrated within micro- or nanochannels represents a promising technique for lab-on-a-chip devices to provide label-free sensing. However, deterioration of sensitivity and loss of a steady-state response occur due to the diffusion boundary generated by the channel confinement. To address these challenges, arrays of recessed ring-disk electrodes with nanoscale spacing fabricated by multilayer deposition, nanosphere lithography, and multistep reactive ion etching were employed for the electrochemical sensing. The resulting devices were evaluated in generator-collector mode by holding the ring electrodes at a constant potential and performing cyclic voltammetry by sweeping the disk potential. Steady-state response and enhanced faradic current (~10x) were achieved for an array operated in open geometry. The application of the arrays for the analysis of dopamine produced a linear response ranging from 100 nM to 1 mM with a detection limit of 20 nM. These arrays can selectively analyze species based on differences in redox reversibility or redox potential. Using hexaammineruthenium(III) (Ru(III)) and ascorbic acid (AA) as model analytes, the selectivity of Ru(III) against AA improves to ~70 in an array integrated within nanochannels. In addition, AA could depleted by holding the ring electrodes at +0.5 V, allowing determination of dopamine at the disk electrodes in the presence of a 100-fold excess of AA. For electrochemically reversible species, a ring potential can be chosen such that interference exhibits exclusively cathodic (anodic) current, allowing the target analyte to be determined from its anodic (cathodic) current. This approach for selective detection was demonstrated in a mixture of Ru(III) and ferricyanide, and successfully applied to differentiate species with overlapping redox potentials, such as dopamine/ferrocyanide and ferrocenemethanol/ferrocyanide.
In this study, we investigate the effects of current rectification on voltage-driven delivery from nanopipettes. A carbon ring/nanopore ultramicroelectrode (UME) was fabricated from a quartz nanopipette coated with pyrolyzed parylene C (PPC) and further insulated with parylene C. This nanopipette-based UME was utilized to perform the voltage-driven delivery experiments and real-time detection of both positively (FcTMA\(^{\text{+}}\)) and negatively (Fe(CN)\(^{\text{3-}}\)) redox species was achieved. Our results demonstrate that under appropriate electrolyte concentrations, voltage-driven delivery is influenced by concentration polarization at the pipette tip region. As a consequence of concentration polarization, we observe enhanced delivery for negatively charged redox probe Fe(CN)\(^{\text{3-}}\) and diminished delivery for positively charged species FcTMA\(^{\text{+}}\).

**Keywords:** Detection, Electrodes, Electrophoresis

**Application Code:** Other (Specify)

**Methodology Code:** Electrochemistry
Polycrystalline boron doped diamond (pBDD) is a robust electrode material that is known to possess an ultra-wide solvent window in aqueous media, low capacitive currents, and high resistance to fouling and corrosion processes. Most of the above attributes depend on the quality of the diamond electrode (e.g. sp\(^3\)/sp\(^2\) ratio) and its termination.\(^1\) In this study we use all-diamond oxygen-terminated high quality pBDD dual-band structures (Figure 1a) to electrochemically detect hydrogen sulfide ions (HS\(^{-}\)) in aqueous solution, under forced convection conditions. In basic solutions (pH 10) HS\(^{-}\) oxidation proceeds via two electrons resulting in sulfur production (see equation scheme, Figure 1b).\(^2\) When the pH of the sensing solution decreases to more acidic values (pKa 6.9), the oxidation signal diminishes because of the higher fraction of non-ionic H\(_2\)S present in solution. This proof of principle study shows that high pH generation by cathodic polarisation of an upstream pBDD electrode causes a sufficiently basic shift in pH so that the adjacent electrode experiences a signal enhancement due to a shift in solution equilibrium for hydrogen sulfide. In addition, electrode cleaning procedures were developed to compensate for electrode fouling effects.

References

Keywords: Electrochemistry, Electrodes, Flow Injection Analysis, Sulfur
Application Code: General Interest
Methodology Code: Electrochemistry
Measurement of solution conductivity is crucial for many industries and is used e.g. in waste water treatment, desalination, food preparation, pharmaceutical, quality control, analysis, etc. Especially for [i]in situ[/i] use, a desirable quality is a calibration free sensor. For working in challenging environments e.g. high temperature/pressure, acidic/basic, the conductivity meter must also be robust and durable. A conductivity sensor fabricated entirely from diamond meets the required attributes. Here we describe the construction and application of an all diamond conductivity sensor for the determination of conductivity across a range covering up to seven orders of magnitude. The sensor is made from intrinsic diamond, machined to produce trenches of varying thickness and separation, which is then overgrown with conducting boron doped diamond (BDD) and polished flat to produce a co-planar structure comprising BDD bands insulated by intrinsic diamond (Figure 1). Results are presented which demonstrate the workable range, reliability, repeatability, use in extreme environments and for lengthy periods of time, [i]in situ[/i].

Keywords: Electrode Surfaces, Electrodes, Sensors
Application Code: General Interest
Methodology Code: Sensors
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 drawbacks of high cost, limited supply, and susceptibility to poisoning by alcohol fuel. One dimensional metal nanostructures have been studied extensively because of their potential use as active components of interconnects in fabricating electronic, photonic, and sensing devices. As a catalyst, they show better electrocatalytic stability than zero dimensional one, because of the lesser vulnerability to dissolution and aggregation.

In this presentation, therefore, we demonstrate the synthesis and electrochemical characterization of one dimensional nanocomposites stemmed from Ag nanowires as an alternative non-Pt catalyst for ORR. Ag/AgCl and Ag/AgBr nanowires are prepared by galvanic replacement reaction between Ag nanowires and FeCl$_3$ or FeBr$_3$, respectively, due to the relatively lower oxidation potential of Ag. The structure, morphology, composition, ORR activity, and stability of the as-prepared nanocomposites are investigated. In order to understand the experimental results, ORR catalyzed by Ag/AgCl or Ag/AgBr nanocomposites is studied using density functional theory (DFT) calculations.
Electrodes and Electrode Surfaces

The search for new catalyst material systems for high-performance oxygen reduction reaction (ORR) is important due to the wide range of applications for amperometric oxygen sensing, fuel cell, etc. ORR, however, is a sluggish reaction and requires a high overpotential. The best ORR catalyst material today is platinum and its various nanostructures are fabricated and utilized to enhance the catalytic activity via the surface area increase. Scanning electrochemical microscopy (SECM) has been proven to be useful in studying the mechanism associated with electrode reactions. The feedback mode is the main quantitative operation mode of SECM, and the steady-state tip generation/substrate collection (TG-SC) mode appears to be attractive for measuring intermediates of tip electrode reaction. With feedback and TG-SC modes, SECM is well-known for the 99% accuracy by capturing almost all of intermediates generated from the tip electrode through the microscale displacement between the tip and substrate, minimizing experimental errors, such as the effects of the resistive potential drop in solution, the charging current, etc.

In this presentation, highly porous Pt layer with porosity control is electrochemically deposited on Pt disk tip electrode, and the ORR mechanism at this porous Pt in acidic solution depending on the controlled porosity is compared with that at bare Pt microdisk. The feedback and TG-SC modes of SECM are used to characterize the mechanism of ORR occurring on Pt disk tip electrode (10 μm in diameter) with a larger Pt disk substrate electrode (76 μm in diameter). While the applied potential to the tip electrode is swept, a constant potential enough to oxidize ORR intermediate is applied to the substrate electrode to investigate the ORR mechanism depending on the catalysts' porosity. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. 2011-0015619).

Abstract Text

Keywords: Chemically Modified Electrodes, Electrochemistry, Microelectrode
Application Code: General Interest
Methodology Code: Electrochemistry
Can you find the data you need in seconds, regardless of who, what, when, where, why, or how it was generated? Can you click-and-create complex technical reports or large sections of regulatory submissions in seconds? Can you click-and-share data with colleagues in your organization? Or click-and-compare your data with data obtained from your partner organization? Are your laboratory systems smart enough to prevent errors before they occur? Being able to answer "Yes" to these questions is our motivation for building an open framework for laboratory data using common information standards.

This presentation describes our development of a commercial-quality framework in partnership with a professional software company, with funding and support from the pharmaceutical industry. The framework is supplemented with limited feature, proof-of-concept software to demonstrate how future commercial software solutions could use the framework to acquire, record, search for, and automatically archive HPLC-UV data. The framework contains three key components: 1) a common, non-proprietary file format that stores laboratory data in a human-readable format (e.g., AnIML-XML); 2) a metadata repository that ensures accurate, complete, and consistent experimental context is stored with raw data, creating an archive-ready file format; and 3) freely distributable class libraries (software tools) that instrument and software providers can use to create new, innovative solutions using common information standards. We will also discuss how we are evolving iterative solutions using agile development to achieve this vision and to help create an open "ecosystem" for new, innovative software for laboratory informatics. Finally, we will encourage stakeholders in the analytical community to work with us through collaboration, expert advice, and active membership.

Keywords: Laboratory Informatics, Sample & Data Management, Scientific Data Management, Software Application Code: Pharmaceutical Methodology Code: Laboratory Informatics
In an era of increased competition, many organizations would benefit from an increase in efficiency and overall quality of their laboratories. Several candidate areas for improvement to achieve these gains include reduction in manual, paper-based processes, assurance that established SOPs are followed, and automation of data capture and calculations. Selecting the right informatics strategy can help accomplish these goals. One of the challenges when selecting an informatics strategy is that with the maturation of electronic notebooks (ELNs) and evolution of LIMS over the last decade has resulted in overlapping capabilities among LIMS, ELNs, and even enterprise resource planning tools (ERPs). The availability of multiple tools to accomplish the same tasks makes selection of the best solution challenging. Questions arise such as: Can our existing resources be better utilized to meet the needs? Should we bring in an ELN or LIMS system? Do we need to combine multiple solutions? The answers to these questions depend on the specific needs of the laboratory in question. This presentation will outline the relative strengths and weaknesses of ELNs and LIMS and provide guidelines to help determine which system will provide the maximum benefit.

Keywords: Lab Management, Laboratory Informatics, Scientific Data Management, Software
Application Code: General Interest
Methodology Code: Laboratory Informatics
Your organization operates as a complete and integrated system, and your software automation solutions should behave in the same way. The varied activities that comprise your workflows are interrelated and interdependent, and optimizing them in as simple and rational a way as possible is essential to overall efficiency and effectiveness.

The laboratory related processes of a typical enterprise consist of two distinct areas of activity. One concerns the operational aspects of the laboratory including: the complete sample lifecycle, management of resources, monitoring of performance metrics, and the use of data accumulated over time. This essential area is the role of the Laboratory Information Management System (LIMS). The other concerns the capture of structured and unstructured data, as well as the tracking and control of test execution. This essential area is the role of the Electronic Laboratory Notebook (ELN).

These two product segments have evolved independently, and are often thought of as separate and distinct applications that benefit from the implementation of a comprehensive interface strategy. The fallacy of this thinking lies in the fact that such a strategy is highly complex to execute, and even more complex to maintain.

This paper will describe how an Integrated LIMS and ELN Platform Solution eliminates this interface, and how an integrated platform eliminates the cost and expense of such interfaces, while also allowing your organization to standardize on a common enterprise hardware and software platform.

Keywords: Laboratory Automation, Laboratory Informatics, LIMS, Software
Application Code: Laboratory Management
Methodology Code: Laboratory Informatics
A successful implementation of a LIMS, like any other IT project (or any project at all for that matter), requires a laser-like focus on the set of goals that define your project’s success. While gathering requirements and writing functional specifications are the genesis of a LIMS implementation, they are not the implementation itself. However, they do define the parameters of success and once they are agreed upon by the members of the committee, they become the basis of the roadmap to success. Once they are available, the path, cost, and time estimates can be made and this then becomes the map for the implementation.

Doing things right, the first time, is always our goal when beginning a project. However, over 60 of implementation projects fail to reach a go-live status, stay within budget, or meet the deadline. There are many factors that can cause such roadblocks, but there are also ways to avoid these detours. In this presentation, the author will share “Lessons Learned” when implementing a LIMS so you may increase your chances for a successful LIMS Implementation.
On-column focusing of large injection volumes on has long been used as a tool to increase concentration sensitivity and minimize volume overload by creating conditions that lead to high solute retention on the head of the column. This is generally done by injecting the sample in a weak solvent, e.g. water in reversed-phase liquid chromatography. Temperature-assisted solute focusing (TASF) increases retention by leveraging the low thermal mass of capillary columns to cool a small section of the column head to sub-ambient temperatures for short periods of time (ca. <1 minute) prior to rapidly increasing the cooled zone to a higher temperature.

We systematically investigated the efficacy of this approach with capillary reversed-phase columns and a series of paraben solutes. The first 7 mm of the column were cooled (Peltier) for 30 s during the time of the injection while the remainder of the 6-cm column was heated to 60 °C. The cooled zone was rapidly heated to the column temperature after 30 s. For a series of 15 injection volumes in the range of 7 to 165% of the column void volume (640 nL), the cooling approach was shown to reduce the observed peak width for all injection volumes of methyl, ethyl and propylparaben. For example, the width of the ethylparaben peak ($k' = 22.4$ at 5 °C, $k' = 6.0$ at 60 °C) was reduced by up to a factor of 4 in comparison to separations with the whole column at 60 °C, reduction in peak width for butylparaben, $k' = 38$ at 60 °C, under TASF conditions was only observed for injection volumes greater than the column volume.

Application of TASF without the recent increases in pressure limits of capillary pumps would make this method not practical. Cooling just 7 mm of the 6 cm column to -12.5 °C was found to increase mobile phase viscosity significantly resulting in a 65% increase in back pressure. The potential for application of TASF for effective on-column focusing stems its ability to be used independently as well as in conjunction with other forms of focusing, e.g. solvent-based techniques.

**Keywords:** Capillary LC, Chromatography, HPLC, Liquid Chromatography

**Application Code:** Bioanalytical

**Methodology Code:** Liquid Chromatography
We have been developing ca. 4.0 nm nanodiamond-based core-shell particles by alternatively depositing nanodiamonds and an amine-containing polymer (polyallylamine) onto carbon cores. These nanodiamond-based particles have shown promise in separating a number of critical pairs of acidic herbicides and in high pH separations of pharmaceuticals. However, until now, relatively little has been known about the materials used to make these particles. Therefore, a comprehensive study of the nanodiamonds used to make the shells of our particles has been undertaken. These nanodiamonds have been characterized by X-ray photoelectron spectroscopy (XPS), time-of-flight secondary ion mass spectrometry (ToF-SIMS), Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), X-ray diffraction (XRD), electron energy loss spectroscopy (EELS), inductively coupled plasma mass spectroscopy (ICP-MS), BET isotherm measurement, and elemental analysis. For comparison, other nanodiamond samples from other sources have also been characterized by the same methods. These techniques have revealed the elemental compositions of the nanodiamonds, the key functional groups present in them, and their surface areas and particle size distributions. Efforts are being made to apply this understanding to enhance the performance of our core-shell particles, for application in high pressure liquid chromatography (HPLC).

Keywords: Characterization, HPLC, HPLC Columns
Application Code: Other (Specify)
Methodology Code: Liquid Chromatography
There is a growing interest on graphitic materials due to their use in several fields. As sorbent material they have been investigated since long time, on the other hand, the complexity of the retention mechanisms made difficult their systematic use and sometime even disoriented several skilled researchers. In recent times new analytical strategies made their peculiar properties (sometime orthogonal to RP) very attractive for several applications. In particular the spreading use of QuEChERS methods increased the use of such sorbents because their strong affinity toward pigments and large molecules. The aim of this study was the systematic (as far as possible) investigation of the potentialities of graphitic sorbents, pointing out misinterpretation and incorrect evaluations as well as enlighten their unique features. The evaluated materials (both carbon coated or fully carbon) were deeply characterized and the performances of several different graphitic materials were investigated for their use as sorbent in SPE, QuEChERS, and stationary phase in liquid chromatography. Test compounds were used to explore the characteristics and potentiality of different materials in the different dimensions. The peculiarities emerged in the experiments confirm potentialities and critical aspects. Advantages and drawbacks for each material are reported.

Keywords: Chromatography, Liquid Chromatography, Sample Preparation, Solid Phase Extraction
Application Code: Environmental
Methodology Code: Liquid Chromatography
The past decade has seen substantial improvements in both chromatographic packing materials and instrumentation, resulting in faster and more efficient chromatographic separations. The once common 4.6 x 150 mm column, packed with 5 [micro]m particles, has been replaced by smaller dimension columns packed with highly efficient materials. However, even today, the use of sub-2- [micro]m particles packed into 2.1 x 50 mm columns remains a challenge. Often the actual performance of these highly efficient materials is lower than what is achievable for these materials. The key is to understand the role that the chromatographic system plays on the ultimate column performance achievable from the sub-2 [micro]m packings. To maximize the performance from highly efficient materials packed into 2.1 mm i.d. columns, it is imperative that the chromatographic system also be optimized with the lowest possible system volume. The objective of this investigation is to characterize the dispersion of three chromatographic systems and demonstrate their impact on demanding applications using 2.1 mm and 3.0 mm i.d. columns packed with sub-2- [micro]m solid-core materials. Chromatographic examples will be shown that illustrate the performance that is achievable with a chromatographic system that has been optimized and one that has not been optimized for use with 2.1 mm i.d. columns.

Keywords: HPLC, HPLC Columns, Instrumentation, Liquid Chromatography
Application Code: Pharmaceutical
Methodology Code: Liquid Chromatography
Diabetes mellitus is a metabolic disease resulting from unregulated glucose homeostasis. The combination of insulin and glucagon and other hormones secreted from islets of Langerhans are of great interest to better understand blood glucose regulation. To delineate the secretion patterns of these peptides to help understand how they go awry in diabetics, it would be ideal to have an automated method that monitors secretion of multiple peptides from islets.

To achieve this goal, a multiplexed competitive immunoassay was developed to measure insulin, glucagon, and islet amyloid polypeptide (IAPP) secretion from batches of islets on a microfluidic device. The labeled antigens, antibodies, and antigens were electrophoretically sampled from their respective reservoirs, mixed, and injected into a separation channel for quantitation every 12 s. A two-color detection system was used to spectrally separate the unresolved bound peaks and detect analytes with large differences in secreted concentrations from islets.

Online immunoassay calibration curves were established based on the bound to free ratios (B/F) for different concentrations of unlabeled antigens. B/F stabilized within 5 min with RSDs < 4%. Detection limits were 20 nM for insulin, 2 nM for glucagon, and 1 nM for IAPP.

The multi-hormone secretion profiles from islets in response to glucose challenges were monitored and showed glucose-dependent patterns of peptide release. This is the first simultaneous automated monitoring of insulin, glucagon, and IAPP secretion and will be useful for understanding secretion dynamics from normal and diabetic islets.
Microfluidics: Monitoring and Multiple Analytes

Molecular Detection Utilizing Surface-Plasmon-Assisted Fluorescence in a V-Shaped Microfluidic Channel

Fluorescent labeling is a powerful technique for high-sensitivity detection of biomolecules. It has been well known that surface plasmon resonance (SPR) enhances fluorescence intensity and the enhanced fluorescence is frequently used as a mean of detecting small amount of biomolecules labelled with fluorescent dyes. SPR can be induced under the Kretschmann configuration using a sensing plate composed of a thin metal film and a transparent substrate, where the transparent substrate is attached to an optically matched prism. A flow channel is often set up on the metal film of the sensing plate. Thus, the prism, the sensing plate, and the flow channel are discrete items, which should be attached and detached in each independent experiment. Also, the optical setup is complicated by the use of a prism, as it has to be angled appropriately to ensure the optimum irradiation of incident light. These facts limit the range of applications of surface plasmon-based sensing devices.

In the present research, we developed a simple, highly sensitive optical sensing system in which SPR enhanced fluorescence. Instead of the prism, “V”-shaped fluidic channels were employed, which functioned as the prism and flow channel simultaneously. Superior performance was demonstrated for biomolecular recognition reactions on a self-assembled monolayer, and the sensitivity reached 100 fM with biotin-streptavidin interactions. In addition to the prism-free configuration, the developed system has an advantage of reduced sample consumption (1–2 µL), making this platform suitable for use in the case of low sample volume.

Keywords: Biosensors, Detection, Fluorescence, Immunoassay
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Microfluidics: Monitoring and Multiple Analytes

A PDMS/Paper Hybrid Microfluidic Biochip Integrated with Graphene Oxide-Based Nanosensors for Multiplexed Pathogen Detection

Infectious pathogens often cause serious economic loss and public health concerns throughout the world. Herein, we have developed a polydimethylsiloxane (PDMS) /paper hybrid microfluidic system integrated with aptamer-functionalized graphene oxide (GO) nano-biosensors for simple, one-step, multiplexed pathogen detection (Figure 1). Each device substrate has its own advantages and disadvantages. Although enormous PDMS-based or paper-based microfluidic devices have been developed, PDMS/paper hybrid systems that take advantage of both substrates are rarely reported. As far as we know, this might be the first PDMS/paper hybrid microfluidic system integrated with nanosensors. The novel use of the paper substrate used in this hybrid microfluidic system facilitated the integration of aptamer biosensors on the microfluidic biochip, and avoided complicated surface treatment and aptamer probe immobilization in a PDMS or glass-only microfluidic system. Lactobacillus acidophilus was used as a bacterium model to develop the microfluidic platform, with a detection limit of 11.0 cfu/mL achieved. We have also successfully extended this method to the simultaneous detection of two infectious pathogens-Staphylococcus aureus and Salmonella enterica (Figure 1c). This method is simple and fast. The one-step ‘turn on’ pathogen assay in a ready-to-use microfluidic device only takes ~10 min to complete on the biochip. Furthermore, this microfluidic device has great potential in rapid detection of a wide variety of different other bacterial and viral pathogens. Financial supports from NIH/NIGMS, UT STARS Award, IDR2 and URI award from University of Texas at El Paso are gratefully acknowledged.

Keywords: Bioanalytical, Biosensors, Lab-on-a-Chip/Microfluidics, Nanotechnology
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Recently, our group has shown the ability of silicon photonic microring resonators to quantitatively detect DNA, miRNA, mRNA, and proteins. This technology is scalable, highly multiplexable (128 sensors/chip), and cheap (<$1/assay). Current nucleic acid analysis methods have distinct disadvantages when compared to microring resonators. qRT-PCR is incredibly sensitive but requires expensive reagents and has limited multiplexing capacity. Conversely, microarrays are multiplexable, but labor intensive and expensive. Here we use the microring resonator platform to quantitate expression levels of multiple mRNAs (20+) relevant to glioblastoma subclassification by way of asymmetric polymerase chain reaction without use of expensive labels.

Our biological system of interest is glioblastoma multiforme, the most common form of malignant brain tumor that accounts for 17% of all primary brain tumors. Despite its prevalence, current treatments result in a median survival time of 15 months, partly due to tumor heterogeneity. Analysis of mRNA and miRNA expression profiles in patient tumor samples has identified 5 different subclasses that are genetically and clinically distinct. A panel of miRNA, mRNA, and proteins has been identified as predictive of different subclasses due to their role in neural growth and differentiation. These subclasses have also lead to different median survival times. The aim of this abstract is to validate a platform to probe these biomolecular panels due to the current lack of cost-effective technologies that can be used for quantitative detection of multiple biomolecules in a clinical setting.

The National Institutes of Health and the NIH-sponsored Midwest Cancer Nanotechnology Training Center support this work.

Keywords: Bioanalytical, Biosensors, Nanotechnology, Nucleic Acids
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Organs-on-chips 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. In order to maximize the insights gathered from these systems, it is advantageous to multiplex microscopy, electrochemical monitoring, and online ion mobility mass spectrometry (IM-MS) to monitor cellular morphology and metabolic flux of small molecules and proteins over time into one platform. The Perfusion Controller/ Microclinical Analyzer (PC/[micro]CA), a stand-alone perfusion controller with continuous on-line analysis and calibration, has been assembled from commercially available microfluidic syringe pumps, valves, and reservoirs from Labsmith, Inc. The PC/[micro]CA consists of eight pumps and seventeen valves, each monitored and controlled through a customizable graphical user interface (GUI). The GUI enables complex programming capable of running for days at a time, including automated cell loading, continuous perfusion of media, calibration of the sensor array, and the inclusion or exclusion of any of the measurement methods. The PC/[micro]CA is assembled on a printed circuit board designed to facilitate housing within an incubator, as well as on a Nikon Ti Eclipse microscope, where the bioreactor can be interrogated with optical methods. The PC allows for a bioreactor to be loaded with cells, and continuously perfused with basal media or drug targets. Effluent is then directed to the [micro]CA for metabolite detection with an electrochemical sensor array, followed by injection into an IM-MS. The PC/[micro]CA is highly adaptable, and can be modified to accommodate any bioreactor which can be interfaced with 360 [micro]m tubing. The automation, measurement capabilities, customizability, and abilities for long-term use make the PC/[micro]CA an ideal instrument for high-yield systems biology research. This work funded by DTRA 100271 A-5196.
Microfluidics: Monitoring and Multiple Analytes

On-Chip Droplet Detection and Quantification - Taking Control of Digital Microfluidics for Chemical Analysis

Droplet microfluidic systems have advantages on-chip of reproducible generation and handling of small samples and fast mixing of added reagents within an inert encapsulated environment. Analytically, this has led to widespread applications in chemistry, biology and medicine.

Optical detection remains the dominant detection mode in the field. Superb spatial and temporal resolution (using high frame-rate cameras) and sensitivity (using laser induced fluorescence) has made optical detection highly desirable in a research environment, while the different phases in segmented flow system has hindered the use of detection methods dependent on surface chemistry. However, due to the costly and bulky nature of optical detection, there is a need for a miniaturized, integrated detection methods.

We present here a miniaturized device allowing reliable in-line droplet detection in water-in-oil segmented flow. Our device can detect droplets reliably, as well as probing characteristics of the segmented stream, such as velocity, droplet volume, dispersity, and duty cycle.

The contactless nature of our device has not only eliminated contamination and electrode surface passivation, but also leaves the chemistry of the droplet undisturbed, allowing direct coupling with other analytical techniques to quantify droplet contents. Furthermore, the output signal can be used for downstream manipulation of droplets, such as droplet sorting, dosing and control of valves.

Keywords: Detector, Lab-on-a-Chip/Microfluidics, Microelectrode, Validation

Application Code: Bioanalytical

Methodology Code: Microfluidics/Lab-on-a-Chip
A multichannel linear-array graphene oxide (GO) aptasensor supported on a solid surface was successfully demonstrated. We realized this sensor by modifying the GO surface with an originally developed pyrene-aptamer-dye probe. Each segment works as a linker to the GO surface, a protein recognition part, and a fluorescence detection tag, respectively. We have achieved a diagnosis level of the detection limit for thrombin with this system. The system allows us to perform molecular detection on a solid surface, which is a powerful tool to realize an on-chip sensor, especially to form a micropattern of the probes. A merit of using a multichannel and/or microarray configuration is that it facilitates a quantitative comparison of several different measurements.

In this work, we formed a linear array of two different probes, which contains aptamers for different targets, thrombin and prostate specific antigen (PSA), on the GO surface firmly fixed on a glass support. We then implemented a polydimethylsiloxane sheet with a dual-channel microfluidic device across the array to prepare a multichannel linear-array GO aptasensor. We measured the fluorescence image of this sensor after injecting thrombin and PSA solutions into the top and bottom microchannels, respectively, by using a confocal laser scanning microscope. The bright fluorescence was observed only in the corresponding areas where the correct aptamer-target pair supposed to be formed. A simultaneous detection of multiple target molecules on a single chip was successfully demonstrated. Our findings will advance the field of on-chip microsensors.

Keywords: Biosensors, Fluorescence, Protein, Sensors
Application Code: Bioanalytical
Methodology Code: Microfluidics/Lab-on-a-Chip
Recently, D-forms of amino acids (AAs) have attracted the attention of researchers due to their biologically important functions. As one example, D-Aspartate in the neuroendocrine system is known to behave like a hormone to regulate the steroid hormone synthesis in the rat pituitary gland. D-Serine appears to be the endogenous ligand for the glycine binding site of the critically important NMDA receptor. In addition, other D-AAs have been reported to be present in endocrine and neuronal tissues, including D-Glutamate, D-Proline and D-Alanine. In this way, the unusual chiral forms of common compounds appear to play important roles in the nervous system, especially as neurotransmitters. Further understanding the role of chirality in intercellular signaling is essential, but a lack of the appropriate analytical technology has hindered investigations of this key molecular property.

For clarifying the presence and how D-AAs work in the biological system, capillary electrophoresis (CE) is an excellent separation method due to its high separation performance and low sample consumption. In this study, we couple CE with laser-induced fluorescence detector to quantify small amount of D-AAs. For enantioseparation, we added chiral selectors such as quaternary-amino-[beta]-cyclodextrin and hydroxypropyl-[gamma]-cyclodextrin to the electrolyte. Combination of CE with mass spectrometry (MS) provides greater assurance for qualitative analysis. Special separation conditions have been designed to avoid the introduction of chiral selectors to the mass spectrometer. Sensitivity improvement by an online sample concentration technique was also studied to provide reasonable detectability of D-AAs. These techniques have been applied to analyze D-AA content in neuronal tissues.

Keywords: Bioanalytical, Capillary Electrophoresis, Mass Spectrometry, Neurochemistry
Application Code: Bioanalytical
Methodology Code: Capillary Electrophoresis
Changes in neurochemical signaling are thought to be important for the development of psychological disorders and addictions. Of particular interest is the catecholamine norepinephrine, whose actions are implicated in integrating stressful and aversive stimuli. Norepinephrine is an easily oxidized neurotransmitter found most concentrated within the bed nucleus of the stria terminalis (BNST). Norepinephrine modulation within the BNST is thought to play an important role in the development of drug addiction, and we have previously demonstrated how release and regulation in the BNST change in response to morphine dependence in anesthetized rats. New advances in voltammetric acquisition software now allow us to make measurements from minutes to hours. Here, we use this advantage to monitor the neurochemical changes that occur during withdrawal in awake rats <i>in vivo</i> and in real time. Freely moving rats are forcibly withdrawn from morphine by administration of the opioid receptor antagonist naloxone, and somatic indices of withdrawal are correlated with NE and pH changes in the BNST. The ability to quantitate subsecond events over longer periods of time will aid in our understanding of norepinephrine’s involvement in the progression of drug dependence. This mechanistic insight may reveal useful interventions applicable for human populations.
met-Enkephalin (mENK), an opioid peptide in the brain, has been implicated as an important component in the development of learned-behaviors and motivation. Chromaffin cells, found in the adrenal medulla, are also known to secrete mENK along with various other neuropeptides. To date, the analysis of mENK has been limited to chromatographic approaches and various static assays, such as immunoassay. These techniques offer excellent chemical selectivity and sensitivity, but are limited in their ability to detect rapid molecular fluctuations. We have developed and optimized a voltammetric waveform for selectivity, sensitivity, and reproducibility in the electrochemical detection of mENK using fast-scan cyclic voltammetry (FSCV) and carbon-fiber microelectrodes. This approach enables mENK to be distinguished from other endogenous enkephalins and opioid peptides. We have used electrical stimulation of rat adrenal tissue to elicit peptide release, and monitored the molecular dynamics with FSCV. HPLC was utilized as a standard against which to compare voltammetric results. This work reports the first known instance of sub-second detection of endogenous mENK in living tissue, and lays the foundation for future in-depth studies using this real-time approach.
Cerebral blood flow (CBF) serves to supply the brain with glucose and oxygen to meet metabolic demands. During times of increased neuronal activity, neurotransmitters regulate local blood flow by influencing vessel dilation. Histaminergic neurons are well-positioned to influence CBF as its neurons broadly project throughout the brain and its receptors are located on both endothelial cells and astrocytes. However, the actions of histamine as a vasomodulator are primarily characterized in the periphery, while much remains unknown regarding how its release affects cerebral perfusion.

To determine how histamine regulates oxygen dynamics in the nucleus accumbens (NAc) of the anesthetized rat, fast-scan cyclic voltammetry (FSCV) at carbon-fiber microelectrodes was utilized. With this technique extracellular oxygen changes, which are indicative of blood flow, were measured as reduction currents at the carbon fiber. Previous work demonstrated that electrical stimulation of the medial forebrain bundle induces a biphasic oxygen response in the NAc. Histaminergic fibers also course through the medial forebrain bundle. It is therefore possible that histamine release contributes to the hemodynamic changes found with this stimulation. To identify the role of histamine in the oxygen response, drugs specific for its receptors (H₁R, H₂R, H₃R) were administered by systemically, by intraperitoneal injection, and locally, by iontophoretic ejection. Comparing results from these two methods allowed discrimination between peripheral and local receptor effects. Since CBF regulation is primarily attributed to glutamate neurotransmission, iontophoretic ejections of glutamate were compared to histaminergic results.

Keywords: Electrochemistry, Microelectrode, Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
Using matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) and multivariate statistical analysis, we identify peptide markers in the brain altered by re-exposure to a drug-paired context to better understand how adaptations caused by exercise alter conditioned contextual drug associations. Previous studies in C57BL/6J mice have shown that exercise after conditioning lessens the preference for a drug-paired context. Here, mice are conditioned to associate a particular floor texture with cocaine and an alternate floor texture with vehicle (saline). After conditioning, half of the animals had access to an exercise wheel for 30 days while the other half remained sedentary. Prior to sacrifice, each mouse was re-exposed to a context that was previously exclusively paired with cocaine or paired with the vehicle, creating four behavioral phenotypes. Tissue extracts were taken from the amygdala and the hippocampus of each animal and pooled by behavioral group. Peptide profiles for pooled samples from each brain region were collected via MALDI MS. Using principal component analysis, mass peaks that were statistically different between behavioral groups were determined. Our initial results suggest a change in peptide levels with re-exposure to a drug-paired context in the hippocampus of animals that exercised after conditioning. In sedentary animals, peptide expression changes were seen in amygdala with re-exposure to the cocaine-paired context. Results are being verified using an alternative approach involving stable isotopic labeling and liquid chromatography coupled to mass spectrometry, and unknown peaks are being identified. This work is funded through NIH and NIDA award number P30 DA018310.
Electrochemical monitoring of adenosine using fast-scan cyclic voltammetry (FSCV) has become increasingly popular due to excellent temporal resolution and relatively good selectivity. Adenosine is the major metabolite of ATP and is involved in a variety of modulatory cascades and brain diseases. Four known adenosine receptors exist in the brain, but A1 and A2A are most abundant. Adenosine binds to inhibitory A1 receptors to cause down-regulation of other neurotransmitter signals. Traditionally, adenosine signaling was characterized on a slow time scale, ranging from minutes to hours; however, recently fast changes in adenosine were characterized. To determine the extent to which adenosine modulates neurotransmitters on a fast time scale would be beneficial to understand the function of rapid adenosine signaling. We have developed a method to characterize adenosine modulation of dopamine using FSCV in the rat caudate putamen by exogenously applying adenosine using a picospritzer and then monitoring electrically stimulated dopamine release. Time between exogenous application and stimulation was varied and increasing time provided less of an effect on dopamine release. Stimulated dopamine release decreased by 50% after transient adenosine application, indicating an inhibitory modulation. Stimulated dopamine release recovered between 10 and 20 minutes after application. Adenosine receptor antagonists will be given to determine which adenosine receptor is involved in dopamine modulation. This is the first time to show that adenosine transiently inhibits dopamine release and the affect lasts only a few minutes. The discovery of rapid modulation of dopamine release by adenosine is beneficial for development of adenosine therapeutics for Parkinson’s disease.

Funded by NIH 1R01NS076875

Keywords: Biosensors, Electrochemistry, Microelectrode, Neurochemistry

Application Code: Neurochemistry

Methodology Code: Electrochemistry
Traditionally, adenosine is recognized as a slow acting molecule that regulates physiological processes on a long time scale of minutes to hours. Recently, our lab used fast-scan cyclic voltammetry to measure adenosine on a more rapid time scale of seconds. Using this technique, our laboratory discovered spontaneous, transient adenosine release for the first time in vivo of the rat brain. Here, we studied spontaneous transient adenosine release to better understand the mechanism of release and clearance of rapid adenosine modulation. Using carbon-fiber microelectrodes, we probed spontaneous adenosine release before and after drug administration. NBTI (S-(4-Nitrobenzyl)-6-thioinosine) was used to block equilibrative nucleoside transporters. The duration of release significantly increased from 3.2 to 4.1 seconds following NBTI administration, while the frequency and concentration of adenosine did not change. These results indicate that spontaneous transient adenosine is cleared from the extracellular space through equilibrative nucleoside transporters but is not released through transporters. EHNA (erythro-9-(2-Hydroxy-3-nonyl) adenine), an adenosine deaminase inhibitor, also significantly increased the duration of adenosine release from 2.7 to 3.6 seconds. The EHNA and NBTI data, indicates that adenosine is cleared by transporters as well as adenosine metabolism. ABT-702, an intracellular adenosine kinase inhibitor, was administered to better understand the source of spontaneous adenosine release. ABT-702 does not increase the frequency or concentration of transient adenosine release, suggesting adenosine is not being released from free intracellular sources, but possibly pre-packaged vesicles. These studies reveal a better understanding of transient adenosine release and how to regulate them in the future.

Funded by NIH1R01NS076875.

Keywords: Bioanalytical, Electrochemistry, Microelectrode, Voltammetry
Application Code: Neurochemistry
Methodology Code: Electrochemistry
It is well known that current histological analysis based on antibody-dye immunostaining suffers from difficulties in multiplexing due to spectral overlaps in optical signals. We have recently demonstrated the potential of using MALDI-IMS in clinical histology application by targeting carbohydrate biomarkers. Such mass spectrometric methods offer the possibility of analyzing multiple biomarkers in a single run. Herein we report our progress on the design, synthesis, and imaging studies using trityl-based MS-tag conjugates for cell surface antigen recognition.
Haloacetic acids (HAAs) are regulated and commonly present in drinking water distribution systems. The HAAs form from the reaction of free available chlorine with natural organic matter present in the source water. A recent chemical survey determined that HAAs were also present in all bulk sodium hypochlorite solutions (bleach feedstock) analyzed at the mg/L level. This leads to a situation similar to chlorate and perchlorate ions: the HAAs in the bulk hypochlorite solutions are dosed into the distribution system from the hypochlorite solutions.

The goals of the research were to determine how fast the HAAs formed in the bleach feedstock and the stability of the HAAs in the bleach feedstock. Stability studies were performed by splitting the bleach feedstock solutions and then storing them at temperatures ranging from 35 to 50 [degree]C. The HAAs in the bleach feedstock at each temperature were analyzed over time using post-column reaction-ion chromatography with nicotinamide fluorescence. The formation of the HAAs in bleach feedstock was observed by collecting on-site generated bleach feedstock and immediately placing it in dry ice and water ice. These two temperatures provided a basis for the quickness of HAAs formation in the bleach feedstock. The procedures for collecting bleach samples, analysis for free available chlorine, and analysis of HAAs will be presented. In addition, the results of the temperature studies will be presented that detail the formation and stability of HAAs in bulk sodium hypochlorite solutions.

Keywords: Contamination, Environmental Analysis, Ion Chromatography, Trace Analysis
Application Code: Environmental
Methodology Code: Liquid Chromatography
Automating Near Real Time Trihalomethane Monitoring and Applications to Water Treatment Process Control

The chlorination of drinking water forms regulated, halogenated disinfection by-products, of which the trihalomethanes (THMs) are a major class. The maximum contaminant level for Total THM concentrations in drinking water is 0.080 mg/L set by the United States Environmental Protection Agency (USEPA). Water treatment plants have residual chlorine, total organic carbon, turbidity and pH meters to continuously monitor these parameters. Short term changes in these parameters can result in short term variation of the THM concentrations in finished drinking water, but utilities do not have a “THM meter”. For many utilities changes in water quality parameters can mean the difference between being in and out of compliance for THMs. The USEPA methods are impractical for long term, 24/7 monitoring of THMs, due to their cost and complexity, and have not been widely used to continuously monitor THM concentrations in either a short- or long-term timeframe.

The capillary membrane sampling gas chromatography (CMS-GC) system can be used as a “THM meter” for water utilities. The CMS-GC is an on-line, process monitoring instrument that conducts all sampling, analysis and reporting of individual and Total THM concentrations. Research will be presented where the CMS-GC was used to measure hourly THM concentrations in several unique distribution systems over extended periods (greater than 1 month). The THM data sets are synchronized with water quality parameters routinely measured by treatment plants to determine the relationship between short term variation of water quality parameters and short term variations in THM concentrations within each distribution system.

Keywords: Gas Chromatography, On-line, Process Monitoring, Water
Application Code: Environmental
Methodology Code: Gas Chromatography
Clean water is essential for human life and the purification of water is a costly procedure to maintain our sanitary water source. As a way to save money and in an effort to be environmentally friendly, water purification is looking to plants as the answer to society’s water purification needs. Plants are consistently being researched as away to remove heavy metals and toxins from the environment as a form of phytoremediation. However, as technology advances to use nanoparticles in everyday materials, the understanding of the effectiveness of plants for the removal of these nanoparticles from contaminated waters is lacking without further research. Silver has been used as a useful way to purify drinking water with its antibacterial and antimicrobial abilities. This precious metal while effective at cleaning a water source can actually be quite toxic in higher concentrations. With silver nanoparticles becoming a popular way to coat clothing and home appliances, these nanoparticles enter the water ways through the wash. The tiny sizes of these nanoparticles make them more difficult remove with normal filtration systems. This research looks into the effectiveness of water lettuce in the purification of contaminated waters and health of the plants in environments containing silver(I) and silver nanoparticles in a controlled water source as well as a strontium rich environment utilizing ICPMS for identification of the location of silver within the plant and its water source. This presentation will also touch on the disposal of these plants after their use for water purification.

Keywords: Environmental/Water, ICP-MS, Nanotechnology, Water
Application Code: Environmental
Methodology Code: Mass Spectrometry
Detection of trace amount of pharmaceuticals in water is always a problem. Expensive and less readily available LC/MS and cumbersome derivatization steps in GC/MS make it more difficult. A new, fast and economical HPLC method was developed for the analysis of carbamazepine, venlafaxine and fluoxetine in water samples. A gradient reverse-phase HPLC assay was used with UV and fluorescence detector. Sample was passed through Gemini C18 110A (250x4.60 mm, 5 μm, Phenomenex) column at a flow rate of 1.0 ml/min. A mixture of citric acid (100mM) and EDTA (10mM) was mixed (pH adjusted at 4.5pH by 0.1M NaOH) in water and was used as a solvent A. Mobile phase was made by mixing solvent A and methanol (20:80, v/v). 4L of water samples were collected and separated by solid-phase extraction (SPE) procedure, using the (Oasis HLB, 30 μm) cartridges on a Vac Elut apparatus. Elution was done my 1 ml of methanol (3 times) and evaporated up to 600 μl. 3 HPLC run was done at an injection volume of 100µl. From spiking experiments, limit of quantification (LOQs) for carbamazepine was and 10 ng/L, venlafaxine 1 μg/L and fluoxetine 100 ng/L respectively. HPLC can be used to detect the trace amount of pharmaceuticals in water. The technique requires no derivatization steps, consumes less time and is cost effective.
We report, for the first time, a simple yet versatile platform for the construction of Drug-DNA adducts (DDA), inspired by natural adduct formation, for targeted cancer therapy. In DDA, one ssDNA was covalently conjugated with multiple copies of drugs, while maintaining DNA functionalities, including specific aptamer recognition and DNA hybridization. DDA formation was verified via ESI-MS, gel electrophoresis, and optical spectrometry. DDA was resistant to enzymatic degradation, providing a new strategy to combat nuclease degradation in DNA-based drug delivery. DDA was also stable at relatively low temperature, but it was able to release drugs at physiological temperature. Drug-aptamer adducts maintained the nucleic acid functionality of selective recognition to target cancer cells, as verified by flow cytometry. Confocal microscopy studies showed that DAAs delivered drugs into cells, resulting in drug accumulation in the nucleus, and the selective cytotoxicity of drugs delivered by DAAs was demonstrated using an MTS assay. DDA formation was widely applicable. Indeed, two other drugs and a variety of ssDNAs were also capable of forming DDAs, without modification of extra DNA sequences for drug conjugation. Our preliminary study showed that the adduct reaction occurs mainly on Guanine, providing insight into future DNA design for the development of DDA-based targeted drug delivery systems. The nanostructure building blocks used to prepare DDAs retained the ability to assemble nanostructures which could be loaded with therapeutics ready for targeted drug delivery. The in vivo evaluation of a drug-aptamer adduct has further validated DAA for targeted cancer therapy, in which drugs showed potent antitumor efficacy with reduced side effects compared to free drugs. These remarkable features make this DDA platform promising for broad application and production scale-up in the development of DDA-based target drug delivery systems for cancer therapy.

**Keywords:** Biomedical, Biopharmaceutical, Biotechnology

**Application Code:** Biomedical

**Methodology Code:** Chemical Methods
Phytosterols are a group of naturally occurring steroid alcohols found in plants. They are key structural components of plant cell membranes, assuming the role that cholesterol plays in mammalian cells. There is considerable interest in phytosterols as dietary supplements as they are reported to lower cholesterol levels and also have a positive impact on cardiovascular diseases. However, recent research suggests that phytosterol supplementation may aggravate atherosclerosis and lead to aortic valve stenosis. Phytosterols are typically measured by gas chromatography (GC). However, this approach is time-consuming since it requires saponification of the sample, several extractions, and followed by derivatization. Here, a simplified method is presented using reversed phase, high-performance liquid chromatography (HPLC) and electrochemical detection using a boron doped diamond electrode.

Samples were prepared for HPLC analysis by saponification with potassium hydroxide to reduce acylglycerols to fatty acids prior to analysis. Five standards campesterol, cholesterol, stigmasterol, beta-sitosterol, and stigmastanol were eluted in < 10 min. The LOD was 1 ng on column for all analytes. The method presented here was used to examine the phytosterol content of hydrolyzed red palm oil and hydrolyzed krill oil. The HPLC method with electrochemical detection is simple to implement, has good linearity and sensitivity, and is capable of measuring numerous phytosterols in plant extracts. This approach can be used to examine product purity, supplement content, and adulteration.

Keywords: Analysis, Electrochemistry, Liquid Chromatography, Natural Products
Application Code: Other (Specify)
Methodology Code: Liquid Chromatography
Nicotine has long been recognized as the primary addictive component in cigarettes and other tobacco products. Tobacco industry research revealed that the proportion of the unionized, “free-base” form of nicotine in cigarette smoke correlated well with “smoke pH” and with smokers’ overall satisfaction of tobacco smoke. Since this industry data became publicly available, research has sought to determine free-base nicotine delivery in cigarette smoke, and its variation among different cigarette design features. However, dynamic acid/base and distribution equilibria present significant challenges and limitations in differentiating between the fractions of the pH-dependent forms of nicotine in cigarette smoke. Historically, methods developed to evaluate free-base nicotine have aimed to measure it in both the gas and/or particle phase of cigarette smoke. Modern methods, however, have relied on the volatility and gas-phase partitioning of free-base nicotine from trapped smoke particulate matter. The present research used both solid-phase microextraction and headspace-gas chromatography-mass spectrometry techniques to analyze machine-smoked reference and select U.S. brand cigarette smoke samples under various smoking conditions. Isotopically-labeled nicotine spiked onto smoke samples provided an alternate means to evaluate the effects of brand-specific smoke matrices and/or design features on free-base nicotine determinations. Aqueous and organic solvent-based nicotine solutions were also analyzed to evaluate changes in distribution equilibria. The results of these experiments suggest analytical limitations in volatility-based methods for assessing free-base nicotine cigarette smoke deliveries and highlights the analytical challenges faced in making this determination.
Dissipative structures have the ability (a) to increase and store information in the form of increasing levels of internal structuration, and (b) to export disorganization or entropy to their immediate environment. Eventually, such structures are grounded in the dynamics of self organization. Self-organization could cover structural and dynamic order of both equilibrium structure and non-equilibrium, incorporating non-linear chemical processes and energy flow concerning dissipative structures.

Non-linear phenomena such as temporal oscillations and spatial pattern formation have been described in many of Nature processes. They are very complex which makes the substantial understanding of their mechanism pretty pain. Molecular self-assembly, a concept central to Nature’s forms and functions, is an important route towards the construction of artificial molecular-level machines. Porphyrin derivatives play an important role in Nature processes. There has been, though, denying porphyrins are applied in nonlinear dynamics. The Belousov-Zhabotinsky (BZ) reaction BZ reaction has been harnessed as the driving force of self-oscillation of artificial Nature processes. The tris (bipyridine) ruthenium complex only, though, is applied as the conventional self-oscillation system. In this light, a water soluble porphyrin derivatives built in chemical oscillation was prepared and this novel corresponding system showed self-oscillating reduction and oxidation reaction. This presentation is concerned with self-oscillation driven a water-soluble metallo-porphyrin derivatives for the first time. This system is of interest as a potential ditopic receptor and as a possible disassembly can by promoted by chemical redox system.

Keywords: Biotechnology, Chemical, Nanotechnology, Rheology
Application Code: Nanotechnology
Methodology Code: Chemical Methods
Natural products contain a variety of active components. Many medicines have their origin in natural products and it is said that they account for 50% of the total medicines available. Preparative HPLC is used to obtain active components from complex mixtures such as natural products. In this poster, we will show a preparative separation of rosemary. Rosemary has several beneficial effects such as odor elimination, antibacterial activity, and antioxidant activity, so it has been used for meat dishes in Europe for a long time. Rosemary contains rosmaric acid, carnosic acid, and carnosol as its active components. Rosmaric acid has the ability to control allergic symptoms. Carnosic acid and carnosol exert a revitalizing influence on biological defense mechanisms and enhance detoxification. It is also reported that carnosic acid has the ability to improve memory performance and to facilitate the creation of nerve growth promoting substances which have a significant role in maintaining nerve cells. We used a newly released low-pressure gradient preparative HPLC system in this study. This system enables the combination of four mobile phases, so it is useful for method development. A Shim-pack PREP-ODS column was used for preparative separation and water, methanol and a 2% formic acid water solution were used as mobile phases. As a result, rosmaric acid, carnosic acid, and carnosol were successfully separated from other rosemary components.

**Keywords:** HPLC, Liquid Chromatography, Natural Products, Prep Chromatography

**Application Code:** General Interest

**Methodology Code:** Liquid Chromatography
Comparing Gas and Liquid Chromatography Determinations of Fatty Amines

In contrast to the plethora of publications on the separation and analysis of fatty acids and their esters, analogous studies involving fatty amines are relatively scarce. A recently introduced ionic liquid based capillary column for gas chromatography was used to separate trifluoroacetylated fatty amines focusing on the analysis of a commercial sample. Working at constant 200°C temperature, it was possible to separate linear primary fatty amines from C12 to C22 chain length in less 25 min using mass spectrometry for identification. The log of the amine retention factors are linearly related to the alkyl chain length with a methylene selectivity of 0.117 Kcal mol-1 for the saturated amines and 0.128 Kcal mol-1 for the mono-unsaturated amines. The sp2 selectivity also could be calculated as 0.107 Kcal mol-1 for the IL column. The commercial sample was quantified by GC-FID. Secondary amines were highly retained and tertiary amines were not easily separated by GC since they could not be derivatized. An LC method was developed coupling a C18 column with reversed phase gradient separation with acetonitrile/formate buffer mobile phases and electrospray ionization mass spectrometry detection. Native amines could be detected and identified by their single ion monitoring chromatograms even when partial co-elution was observed. The analysis of the commercial sample returned results coherent with those obtained by GC-FID and with the manufacturer’s technical data.

Keywords: Chromatography, GC, Separation Sciences, Surfactants
Application Code: General Interest
Methodology Code: Gas Chromatography
Cocaine is a popular illegal drug of abuse in the United States. Detection of Cocaine in money has attracted much over the past two decades. Money is contaminated with cocaine; the contamination is transferred to other bills by exposure of contaminated money with non-contaminated money in circulation. Cocaine was extracted using methanol and gas chromatography triple quadrupole mass spectrometer was used for the instrumental analysis. The product ion scan was optimized using 15 eV collision energy and Multiple Reaction Monitoring (MRM) mode was used for MS-MS analysis by selecting 182 m/z as precursor ion and 182 m/z to 82 m/z as the quantitative transition. The method offered high precision with %RSD less than 6%, recovery of 98.3±0.9 % and very low detection limit from standard solutions of 0.005 [micro]g/ml. The method was linear over a wider range from 0.01 [micro]g/ml to 10[micro]g/ml with R2 value of 0.998. US currency with different denominations from different locations were collected and examined for traces of cocaine. Two $ 20 bills collected from Miami showed 1.8 ng and 8.5 ng of cocaine respectively. Two $1 bills collected from New Jersey showed 2.85 ng and 3 ng of cocaine respectively. The developed analytical method using a triple quadrupole mass spectrometer demonstrated high selectivity in detecting trace levels of cocaine in currency.
Chemical, Biological and Explosives Analysis

Retrospective Assessment of Chemical Warfare Agent Exposure in Humans Using LC-MS/MS

Assessing human exposure to chemical warfare agents (CWAs) remains challenging due to low exposure concentrations and complex biomedical sample matrices. We are developing sensitive methods to detect exposure to both nerve and blister agents by analyzing blood samples for highly persistent protein adduct biomarkers. Detection of CWA exposure is critical for both forensic and diagnostic purposes, including investigating incidents of alleged use, administering therapy to casualties, and confirming non-exposure. Advanced sample preparation procedures and mass spectrometric measurements were used to detect in vitro exposure of human blood to sulfur mustard, sarin, and VX. Covalent reactions of agents with nucleophilic sites (amino acid residues containing SH, OH, NH, and CO2-) on proteins (e.g. albumin, cholinesterases) create the most persistent signatures of CWA exposure. Sample preparation methods were optimized for isolation of albumin (sulfur mustard adducts) and butyrylcholinesterase (BChE; sarin and VX adducts) from human blood. Selective enzymatic digestion of the isolated proteins generated peptides, and specific alkylated or phosphonylated peptides were detected using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Synthesized reference compounds, including unlabeled and stable isotope labeled albumin and BChE peptides, sarin- and VX-adducted BChE peptides, and a sulfur mustard-adducted albumin peptide, were used to quantify sample preparation recoveries and LC-QTOF-MS/MS results. Method limits of detection were sensitive enough to monitor realistic exposure levels and will be discussed. Detection of persistent protein adduct biomarkers is a promising approach for assessing and monitoring CWA exposure. Prepared by LLNL under Contract DE-AC52-07NA27344

Keywords: Biomedical, Forensic Chemistry, Liquid Chromatography/Mass Spectroscopy, Peptides

Application Code: Homeland Security/Forensics

Methodology Code: Liquid Chromatography/Mass Spectrometry
In recent years, the impact of primary biological aerosol particles on atmospheric processes has been studied with increasing intensity. Airborne fungal spores are considered as the dominant fraction of bioaerosols in the size range 2–10 micrometer. Recent studies have shown that the concentration of these fungal spores could be quantified using specific biomarkers. A new technique is presented here to investigate real-time concentrations of two biomarkers (mannitol and arabitol) in ambient conditions. This technique is based on the coupling between a Particle-into-liquid-sampler (PILS), a liquid chromatography (LC) and an Electro Spray Ionisation source – tandem mass spectrometer (ESI-MS/MS). Ambient aerosols are collected at 15 LPM in the PILS and sent into a loop of the LC. Injection is performed every 12 min and separation is achieved using a Porous Graphitic Carbon column. Quantification and confirmation of mannitol and arabitol are achieved in negative mode by ESI using some specific m/z transitions in Multiple Reaction Monitoring mode. A limit of quantification below 0.9 ng/m³ is calculated here for each compound. The measurements have been performed in the region of Paris (France) for a period of 2 weeks during July 2013. Comparisons were performed using off-line technique and an alternative analytical protocol using IC-PAD. Comparison with individual fungal spore counting were also performed. The first results show that this new technique can provide real time measurements of airborne fungal spores biomarkers and follow the variability of activity of fungal spore. This work is supported by the CBRN-E R&D research program from CEA, DIM-SEnT and CNRS.

Keywords: Aerosols/Particulates, Electrospray, Liquid Chromatography/Mass Spectroscopy, On-line
Application Code: Homeland Security/Forensics
Methodology Code: Liquid Chromatography/Mass Spectrometry
This investigation has been conducted to study the possibility of stimulating fungi with gamma radiation for biodegradation of Oxamyl pesticides. Fungi strains capable for biodegradation of oxamyl are identified as Trichoderma spp. including T. harzianum and T. viride, Aspergillus niger, Fusarium oxysporum and Penicillium cyclopium. The biodegradation of oxamyl at concentration of 200 mg L\(^{-1}\) in mineral salt medium by Trichoderma spp showed that 49.4% and 52.15% loss in oxamyl within 5 days of incubation by T. harzianum and T. viride, respectively. While after 10 days of incubation by T. harzianum and T. viride strains the biodegradation was 72.5% and 82.05% loss in oxamyl, respectively.

Treatment of fungus with gamma radiation indicated that it has potent effect on biomasses of Trichoderma spp. grown on MSM with oxamyl at concentration of 200 mg L\(^{-1}\) within 7 days of incubation. The biomass of T. harzianum strain was increased significantly at 100 and 250 Gy by 19.7 and 22%, respectively. While the least mycelial dry weight attained at 5 KGy by 49.8% decrease in biomass. As for T. viride strain 27 and 40% increase in mycelial dry weight at 100 and 250 Gy, respectively was resulted. The doses of 1, 2 and 5 KGy showed decrease in mycelial dry weight by 21.8, 27.9 and 38.2, respectively.

Keywords: Biological Samples
Application Code: Bioanalytical
Methodology Code: Chemical Methods
Much attention has focused recently on the detection of chemical warfare agents, especially nerve agents, in the environment due to the threat of their release by terrorist groups. In our laboratory, we have developed a fast, reliable UPLC/MS/MS method for the analysis of nerve agent degradation products of VX, GB, R-VX, GF and GD. The UPLC/MS/MS method is suitable for water, soil and wipes samples, following the appropriate extraction steps, without the need for derivatization. Chromatographic separation is performed using a hydrophilic interaction chromatography (HILIC) technique. Analytes are detected by electrospray ionization followed by tandem mass spectrometry (MS/MS) in positive and negative ion modes. The total cycle time for nine analytes was less than 9 minutes. Average recoveries for the degradation products ranged from 87% to 102%, and MDL values were less than 20 µg/L for waters, less than 30 µg/kg for soils and less than 10 ng/wipe for wipe samples.
Effect of Dopant on the Ion Mobility of Chemical Warfare Agents

In the cases of chemical warfare/terrorism, it is desired to detect the chemical warfare agents (CWA’s) in the field. Portable ion mobility spectrometry (IMS) equipment have been used for detecting hazardous materials in the air directly, which are sometimes adopting dopant system to raise the sensitivity and selectivity for CWA’s detection. We investigated the effect of addition of several dopants on the IMS behavior of various CWA’s. Volatile CWA’s dissolved in n-hexane or gasoline (obtained from local gas station) were vaporized in 500 mL glass container. Hydrogen cyanide and cyanogen chloride were vaporized in 10 L glass container from potassium cyanide by addition of sulfuric acid or chloramine T, respectively. Phosgene and chlorine were prepared by a permeator (Gastec Inc, Japan). The dopant vapors were prepared by diluting the vaporized solvents (acetone, dichloromethane, diethylether) or ammonia gas with the air. The prepared CWA’s vapor, dopant vapor and/or gasoline vapor were mixed, and introduced to the inlet of the portable IMS equipment (MMTD, Smiths Detection, UK, 63Ni ionization, a long drift tube with elevated temperature (around 100°C.), calibrant correction and clean air drift gas circulation), and IMS behaviors were observed. Addition of acetone or diethyether moved the positive reactant ion peaks (RIP) to the direction of low ion mobility. Addition of ammonia moved the positive RIP to the direction of high ion mobility, and also significantly influenced the IMS behavior of nerve gases and blister agents but not of the blood and choking agents. For example, the sarin monomer peak height was considerably increased in the positive ion mode. Gasoline interfered with the IMS detection of tabun, but addition of ammonia suppressed the gasoline interference on tabun peaks.

Keywords: Air, Detection, Environmental/Air, Forensics
Application Code: Homeland Security/Forensics
Methodology Code: Other (Specify)
The threat of terrorism or criminal bombings has become a serious problem worldwide. To maintain a safe society, detection technologies for hidden explosive devices are in high demand. Current explosives trace detectors (ETD) require human operations for swab-sampling of contaminants from surface of baggage, which makes 100% inspection high-cost. Therefore, an auto-sampling ETD will dramatically reduce human operations, which can be applied to all baggages with lower cost. We report preliminary results of an automated particle sampler for baggage screener combined with the ETD. Explosives particles adhering to the baggage are detached by the air jet and introduced into the cyclone-type particle concentrator through the sampling port. 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 of the automated particle sampler using NIST SRM2905, trace particulate explosive simulants. The ng-level of the particle explosive simulants, which included ng-level of TNT, were adhered to a baggage and we observed TNT signal within 5 seconds after the emission of the air jet from the nozzle to the baggage. The collection efficiency of the automated particle sampler, which are defined as the ratio of the amount of the particles collected by the sampler to the amount of the particles initially adhered to the baggage, were 0.4-1%.

This work was supported by the Ministry of Education, Culture, Sports, Science, and Technology ("Funds for integrated promotion of social system reform and research and development").
At Hamburg University of Technology and Airsense Analytic GmbH the novel analysis system “DACHS” was developed. The main application is the detection of toxic gases in major hazmat incidents. HAZMAT-forces like police or fire service use the “DACHS” on-site to detect the sources of harmful substances. In the field the device is faced with various ambient air conditions. The instrument consists of detectors with different technologies, thus forming a heterogeneous array of sensor. Each sensor has a distinct behavior on alternating sample gas conditions. The system utilizes a PID, three MOS gas sensors, one electrochemical cell for formaldehyde, one for phosgene and an ion mobility spectrometer with membrane inlet. In this work, the response on changing humidity is determined for every sensor in the array. In the described experimental, the humidity of different sample gases is set up by a controlled humidifier under laboratory conditions. The influence of the humidity of the sample can be compensated by optimized calibration curves for the sensors. Therefore, the humidity has to be monitored in the field. The poster describes the relationship between sensor responses and sample gas condition for selected substances. The experimental setup and the measurements will be presented. The optimization results will be shown.

Keywords: Array Detectors, Calibration, Instrumentation, Sensors
Application Code: Homeland Security/Forensics
Methodology Code: Sensors
Hazardous substances are a threat to humans, animals and environment. Thus detection, identification and quantification are very important in case of an intentionally or unintentionally release. For this task portable measurement equipment with a quick response while easy-to-use has to be on site. The gas measurement instrument DACHS fulfills these requirements. It is based on a heterogeneous sensor array, which ensures a reliable detection of all common toxic industrials and warfare agents. For an identification and quantification by a sensor system, it is necessary to measure in the dynamic range of the sensor. Too low concentrations will lead to no detection. High concentrations lead to saturation of the sensor signal, which can lead to wrong findings especially regarding its quantification. Very high concentrations can even damage the sensor. The DACHS uses sensors with high sensitivity to detect even very small amounts of gas (below ppm). To expand the dynamic range to higher concentrations, the sensor system inherits a dilution unit. This unit adds cleaned air to the sample gas, which leads to a decrease of measured concentration. A sieve of activated carbon generates the clean air. Knowing the sensor response and the relation between dilution flow and sample gas flow, the real gas concentration can be calculated. The dilution flow is controlled by a pump and a pressure sensor. Measurements with different gases, concentrations and dilutions are presented.

Keywords: Detection, Environmental, GC, Sensors

Application Code: Homeland Security/Forensics

Methodology Code: Sensors
During industrial production or transportation there are several hazardous substances present, which could be released intentionally or by accident. In this case one of the most important task for emergency forces is to detect and identify these often gaseous compounds. The used measurement instruments have to be well designed to fulfill this difficult task. During their development and maintenance it is very important to get a fast and extensive insight of the system behavior. The understanding of system helps to improve and optimize the detection.

This work presents a handheld heterogeneous sensor array with a gas chromatograph. As sensors an ion mobility spectrometer, a photo ionization detector, three different semiconductive sensors and two electrochemical cells are present. By means of this example, it is shown how to describe, evaluate and predict the systems behavior in general.

The instrument is described by a set of several models and simulations. The fluid dynamics of the gas leading system is simulated by the finite elements methods. The model involves flow, pressure, composition of substances etc. for all parts of the system. A gray box model describes the separation of the gas chromatograph. The shape of the peaks is observed and described by a mathematical model. The response times are calculated with Kovacs retention indices. The spectra of the ion mobility spectrometer are recorded for several substances and the specific ion mobility factors are stored. If several substances are present the spectra are superposed. A mathematical model describes the one-dimensional sensors like the photo ionization detector. The height is adjusted by the substances individual response factor.

With this description of system difficulties during detection become easily traceable and the improvements could be investigated before their realization. For training courses the model provides a deep insight of all parts of the instrument and can it show responses.

**Keywords:** Detection, Environmental, GC, Volatile Organic Compounds

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Sensors
The detection capabilities of field-portable explosive detection devices can be based on either the detection of explosive particles or vapor. Explosive vapor detection methods, instrumental or biological, must be capable of detecting highly volatile and semi-volatile explosive compounds, such as triacetone triperoxide (TATP) and trinitrotoluene (TNT), as well as those with extremely low vapor concentrations, such as hexamethylene triperoxide diamine (HMTD) and ammonium nitrate (AN)-based explosives. Detection of the lower volatility explosives tends to be more challenging. Even with state of the art laboratory instrumentation, in these cases, detection is challenging because vapor concentrations of the explosive compound itself are vanishingly small. This detection challenge is particularly problematic as the peroxide-based explosives (i.e. HMTD) and AN-based explosives are often used in improvised explosive devices (IEDs). Their use has gained popularity due to accessibility of ingredients and ease in preparation, and thus detection has become a priority of military and law enforcement communities.

This work focuses on the determination of the chemical vapor signature associated with low-volatility explosive materials, including associated compounds, chemical precursors and degradation products. By elucidating the vapor signature, improved training methods can be developed for both chemical and biological sensors. In this work, the headspace was sampled by several methods including, active sampling onto thermal desorption tubes, whole-air sampling using an online cryo-cooled injection system, and solid phase micro-extraction. Samples were analyzed by gas chromatography / mass spectrometry (GC/MS) with two different column stationary phases. This work has enabled an observation of the degradation of bulk HMTD over time, as well as a determination of key headspace volatiles associated with bulk HMTD and AN-based explosives.

Keywords: Forensic Chemistry, Gas Chromatography, Headspace, Volatile Organic Compounds
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography
GC-MS, GC-TOF-MS and GC-IRD techniques provide complimentary data in the differentiation of regioisomeric and isobaric piperazine drugs of abuse and controlled substances. The mass spectra for regioisomeric and isobaric piperazines are almost identical with no marked differences that would allow for differentiation between these compounds. Perfluoroacylation of the secondary amine nitrogen of these isomeric piperazines gave mass spectra with differences in the relative abundance of some fragment ions but did not alter the fragmentation pathway to provide unique ions for discrimination among these isomers. Gas chromatography coupled with time-of-flight mass spectrometric detection (GC-TOF) provided a means of discrimination among the isobaric piperazines by confirming the elemental composition of the major fragment ions in the mass spectra of these compounds. On the other hand, GC-TOF-MS was not successful in differentiating between regioisomeric piperazines. The vapor phase infrared spectrum obtained by GC-IRD was successful in differentiating among the regioisomeric and isobaric piperazines by the characteristic bands in the region 700 – 1700 cm⁻¹. These techniques have been applied to the analysis of the ring substituted methoxy-, methylenedioxy-, dimethoxy- and bromodimethoxy-, in both the benzyl and benzoylpiperazine series. In addition to that, isotope labeling experiments such as deuterium (D) and carbon 13 (13C) labeling were used to confirm mass spectrometric fragmentation mechanisms that result in the formation of some key fragment ions or to confirm the elemental composition of these fragment ions. The gas chromatographic resolution will be described for these piperazines on a variety of stationary phases.
Beta radiation is an attractive ionization technique for Ion Mobility Spectrometry due to its reliability and its independence from external power supply. Problematic 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. An Electron Beam pumped Excimer Light source (EBEL) was adapted for the use as an ionization source with an IMS. The EBEL consists of an electron gun operated under ultra-high vacuum (UHV). The generated electron beam with a kinetic energy of 12 keV has enough energy to trespass a thin silicon nitride layer, which separates the UHV region from a chamber filled with a noble gas. This chamber is also called the excimer region ($3 \times 10^5$ Pa). The electrons ionize a part of the gas atoms, which recombine to excited atoms and subsequently form unstable excited dimers (excimers). These excimers decay emitting a Vacuum Ultra-Violet (VUV) photon of an energy characteristic for the composition of the excimer. Argon excimers emit 9.8 eV photons upon decay, argon/krypton excimers emit 10.7 eV photons. After passing a VUV suitable window, these photons directly ionize sample molecules with appropriate ionization energy not higher than the photon energy. In an effort to substitute $^{63}$Ni with a non-radioactive electron source, we used this VUV source as ion source for IMS and we were able to obtain selective ion spectra. We also removed the excimer region from the EBEL and guided the electron beam directly to the sample molecules. With the switchable electron source, we were able to obtain similar ion spectra as with a $^{63}$Ni source.

Keywords: Air, Chemical Ionization MS, Detector, Spectrometer
Application Code: Homeland Security/Forensics
Methodology Code: Other (Specify)
The Gas Detector Array GDA2 consists of a combination of an ion mobility spectrometer (IMS), a photoionization detector, two metal oxide sensors and an electrochemical cell. It was developed for the fast identification of toxic industrial chemicals and chemical warfare agents. A special sampling method was developed in order to use this advanced detection technology also for explosives. A new sampling and thermal desorption tool, which can be used together with the portable instrument, will be discussed. The identification and warning capabilities of the system will be shown. An efficient way to sample traces of explosive materials is wiping of a suspect surface. The material which can be used for wiping should trap the particles, should be free of possible contaminants and should not damage the tested surface. The sampled particles have to be evaporated in order to analyze them. This is done by a new developed thermal desorption tool. Because of the small sample volume a transfer without loss is essential. A low dead volume and a sophisticated thermal design are important. To protect the IMS from unintended external contaminations a membrane inlet is used. The low permeability of the explosive compounds demands special considerations for the membrane inlet. During the ionization process (e.g. by Ni-63) both positive and negative ions are generated. IMS detects charged molecules and separates them according to their mobility. In order to detect both types of ions, positive ions for e.g. peroxide based explosives and negative ions for e.g. nitrogen based explosives the IMS utilizes a fast polarity switching between both polarities in 2 seconds.

Keywords: Environmental/Air, Portable Instruments, Spectrometer, Trace Analysis
Application Code: Homeland Security/Forensics
Methodology Code: Portable Instruments
The ability to detect neutral, cationic and anionic inorganic compounds with a wide range of vapor pressures is a challenging task. The difficulties are amplified when trace quantities are available and are dispersed on “real world” surfaces including glass, metal, plastic, wood, soil or sand. Such an analytical challenge is present in the case of inorganic-based improvised explosives, in particular for the detection of ammonium nitrate. The mass spectral detection of low vapor pressure, inorganic-based explosive signatures including ammonium nitrate, chlorate, perchlorate, sugar and the constituents contained within black powder using the method of laser electrospray mass spectrometry will be discussed.

This ambient pressure mass spectrometry technique combines nonresonant, femtosecond laser vaporization with electrospray postionization and time-of-flight mass spectrometric analysis to provide a universal analysis platform. We report the primary and secondary signatures for trace quantities of inorganic explosives using a mixture of complexation agents in the electrospray solvent. The simultaneous detection of vaporized neutrals, cations and anions were enabled in a single measurement through the use of 1,9-nonanediyl-bis(3-methylimidazolium) difluoride (D\(^2+\)), 1,3-bis[6-(3-benzyl-1-imidazolio)-hexyl]imidazolium trifluoride (T\(^3+\)) and 1-monooleoyl-ra-glycerol (monoolein, MO). Monoolein enabled the unique detection of ammonium ion through the generation of secondary signature ions. Finally, an offline classifier for discrimination of the inorganic-based explosives based on the mass spectral signatures will be shown to have high fidelity identification.

Abstract Text

The ability to detect neutral, cationic and anionic inorganic compounds with a wide range of vapor pressures is a challenging task. The difficulties are amplified when trace quantities are available and are dispersed on “real world” surfaces including glass, metal, plastic, wood, soil or sand. Such an analytical challenge is present in the case of inorganic-based improvised explosives, in particular for the detection of ammonium nitrate. The mass spectral detection of low vapor pressure, inorganic-based explosive signatures including ammonium nitrate, chlorate, perchlorate, sugar and the constituents contained within black powder using the method of laser electrospray mass spectrometry will be discussed.

This ambient pressure mass spectrometry technique combines nonresonant, femtosecond laser vaporization with electrospray postionization and time-of-flight mass spectrometric analysis to provide a universal analysis platform. We report the primary and secondary signatures for trace quantities of inorganic explosives using a mixture of complexation agents in the electrospray solvent. The simultaneous detection of vaporized neutrals, cations and anions were enabled in a single measurement through the use of 1,9-nonanediyl-bis(3-methylimidazolium) difluoride (D\(^2+\)), 1,3-bis[6-(3-benzyl-1-imidazolio)-hexyl]imidazolium trifluoride (T\(^3+\)) and 1-monooleoyl-ra-glycerol (monoolein, MO). Monoolein enabled the unique detection of ammonium ion through the generation of secondary signature ions. Finally, an offline classifier for discrimination of the inorganic-based explosives based on the mass spectral signatures will be shown to have high fidelity identification.

Keywords: Forensics, Laser Desorption, Mass Spectrometry
Application Code: Homeland Security/Forensics
Methodology Code: Mass Spectrometry
Advanced forensic applications were explored using the 5975-SMB GC-MS with cold EI. 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 the TAMI software which provides elemental formulae and is automatically linked with the NIST library.

Several forensic applications will be demonstrated including:

A) High throughput forensic analyses with the new Open Probe fast GC-MS that provides real time analysis (20s) with separation and library identification.
B) Very fast universal GC-MS analysis method of illicit drugs (heroin, cocaine) in under 2 minutes chromatography and 3 minutes total analysis cycle time, using flow programming.
C) The analysis of labile peroxide explosives TATP and HMTD will be shown with dominant molecular ions and femtogram range LOD (also PETN, Tetryl and HMX).
D) Pistol oil on hands for forensic linking between a suspect and a given fire arm.
E) Isomer distribution analysis for fuels and oils characterization for improved arson investigations.
F) Nonoxynol-9 condom spermicide oil analysis.
G) Chemical characterization of Cannabis seeds.
H) Unknown sample identification was achieved via the combination of enhanced molecular ions, extended range of compounds amenable for GC-MS analysis and the TAMI software.

Keywords: Forensics, Gas Chromatography/Mass Spectrometry, GC-MS
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography/Mass Spectrometry
A national incident occurred at the Tri-State Crematory in Nobel, Georgia in 2002. Over three hundred bodies that were transferred to the crematorium were dumped on the property. It was later determined that concrete dust was substituted for human cremains and returned to the families of the deceased. The purpose of this study is to develop a method that can differentiate between cremains and concrete through analysis of the metals present in each. Capillary electrophoresis was chosen as for the analysis of trace metals via complexation with 1,2-diaminocyclohexane tetra acetic acid (CDTA). Separation conditions were optimized and the method was validated using stock metals solutions as well as cremains and concrete samples. Results indicate that CE is a promising technique for the analysis of trace metals in cremain samples.
Forensic analysis of fire debris evidence aims to identify any ignitable liquid present, which may indicate an intentional, rather than accidental, fire. To identify the ignitable liquid, fire debris samples are analyzed by gas chromatography-mass spectrometry (GC-MS) and the resulting data are visually compared to data from ignitable liquid reference standards. Multivariate statistical procedures, such as principal components analysis (PCA), have been investigated to increase confidence in the identification. Using PCA, association of simulated fire debris samples to a reference standard of the ignitable liquid standard contained in the debris has been demonstrated. However, successful association requires that the specific standard is present in the data set analyzed by PCA, which may not be practical for forensic laboratories.

In this research, the potential of associating fire debris according to the chemical class of liquid present, rather than to a specific liquid, was investigated. Reference standards characteristic of different chemical classes were firstly developed and simulated fire debris samples were prepared. Standards and samples were extracted using a passive headspace procedure and analyzed by GC-MS. Total ion chromatograms of the standards were subjected to PCA to ensure sufficient discrimination of the class standards. Scores for the simulated fire debris samples were calculated and projected onto the scores plot to filter out the contribution from interference compounds and pyrolysis products present in the debris samples. Association of the fire debris samples according to chemical class was possible and further statistical procedures were used to provide a quantitative measure of the association.

**Keywords:** Chemometrics, Forensic Chemistry, Gas Chromatography/Mass Spectrometry, Statistical Data Analysis

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Chemometrics
Objectives: A new designer drug, 4-methylamphetamine (4-MeAP), is a ring-methylated derivative of amphetamine and has two regioisomers, 2- and 3-MeAP. Due to the requirement of law enforcement, it is often necessary to demonstrate the specific isomer of ring-substituted compound present in forensic samples submitted for analysis. In this study, we present a GC/MS method for the differentiation of regioisomers of MeAP and methylmethamphetamine (MeMA).

Materials and Methods: 2-, 3- and 4-MeAP and 2-, 3- and 4-MeMA were synthesized in our laboratories. Free bases and their TFA and TMS derivatives were subjected to a GC/MS instrument equipped with any one of three capillary columns (DB-1ms, DB-5ms or DB-17ms).

Results: DB-1ms and DB-5ms columns could not separate free bases and TFA derivatives of 2- and 4- positional isomers while the columns separated incompletely their TMS derivatives. On the other hand, the mid-polar DB-17ms column separated free bases, TFA and TMS derivatives of 6 methylamphetamines though peak shapes of the free bases were tailing. The best separation was obtained from the analysis of the TMS derivatives on DB-17ms column. The mass spectra showed little difference between the 3 regioisomers even after TMS derivatization.

Conclusion: Differentiation of regioisomeric methylamphetamines could be accomplished well by GC/MS of their TMS derivatives on the mid-polar capillary column.

Acknowledgement: This work was supported in part 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, the Japanese Government.

Keywords: Derivatization, Drugs, Forensics, Gas Chromatography/Mass Spectrometry
Application Code: Homeland Security/Forensics
Methodology Code: Gas Chromatography/Mass Spectrometry
The chromatically resolved optic microscope (cromoscope) is a recently developed diffraction grating-based instrument for spectral imaging microscopy. Because of its relatively low cost, tunable spatial and spectral resolution, and nondestructive nature, this instrument should be very versatile and applicable in many different fields of science. One such field is forensic science, where distinguishing among metameric samples (samples that can visually appear to be the same color) is a common problem. We have applied the cromoscope to questioned document analysis as an example of how the cromoscope can be useful in this field. Reflectance spectra of a variety of blue pen inks have been collected and compared. We have found linear discriminant analysis to be a very effective method for classifying these spectra so that metameric samples can be accurately identified. Data treatment and the effects of sample aging will be discussed.
Forensic Analysis

A Spectral Matching Algorithm for Raman Spectroscopy

Raman spectroscopy is useful for identifying chemical constituents and is becoming a routine method for on-site identification of samples. Automated protocols for accurately matching a sample spectrum to spectra contained in Raman databases are needed in many application areas including law enforcement, bioanalysis, pharmaceutical science, geoscience, space science, and homeland security. Because Raman spectra can be acquired using a wide range of laser wavelengths and acquisition modalities, spectra recorded for the same sample can vary considerably. Differences in relative band intensities as well as baseline intensities often avert conventional matching algorithms from yielding correct results. In the work presented here, we introduce a robust method for matching Raman spectra to spectra contained in a database. The method is fully automated and removes baseline contributions as well as differences in relative peak heights. A full theoretical treatment is presented along with validation data obtained for a variety of geological minerals at different laser wavelengths. In addition, we also compare the results of this method to established methods based on Euclidean Distance, Mahalanobis Distance, and Cosine Correlation Analysis.

Keywords: Characterization, Chemometrics, Data Mining, Raman
Application Code: Homeland Security/Forensics
Methodology Code: Chemometrics
Color plays a critical role when analyzing natural fibers such as cotton in forensics. Ultraviolet-visible (UV/visible) microspectrophotometry is a non-destructive technique capable of providing objective color measurements on fibers in the form of absorption or transmission spectra. However, forensic fiber examinations are often hindered by spectra with little detail or points of comparison. We have found that first derivative preprocessing can enhance structure in spectra. Samples of reactive, direct, and vat dyed cotton fibers were analyzed and spectra were preprocessed using one of four methods including baseline correction, normalization, first derivative, and first derivative then a Savitzky-Golay smooth. Principal component analysis, followed by linear discriminant analysis was employed to discriminate cotton samples.

Direct dyed fibers exhibited almost featureless and low absorbing spectra compared to those of reactive and vat dyed fibers. As a result, classification accuracies for direct dyed fibers were lower than those calculated for reactive and vat dyed fibers. The results of this study show that first derivative spectra can significantly enhance classification accuracy when analyzing spectra with only subtle features such as those seen with direct dyed cotton fibers. In cases where calculating the first derivative was shown to be beneficial, application of a Savitzky-Golay noise reduction filter turned out to be disadvantageous. No single method was best for all classes of fibers in the study, and the shapes and intensities of the curves are important when determining if calculating the first derivative is auspicious.
In Japan, recent trends have seen wild silk preferred over cultivated silk because of its texture. Some cases of fraud have occurred were cultivated silk garments are sold as wild silk. Samples from these cases, morphological observation using light microscope and polarized microscope have been conducted in forensic science laboratories. Sometimes scanning electron microscopy was also carried out. However, the morphology of silk shows quite wide variation, which makes it difficult to discriminate wild and cultivated silks by this method. In this report, silk discrimination was investigated using conventional instrumental analyses commonly available in forensic laboratories, such as Fourier-transfer infrared spectrometry (FT-IR), pyrolysis-gas chromatography/mass spectrometry (pyr-GC/MS) and differential thermal analysis (DTA). By FT-IR, cultivated and wild silk gave similar infrared spectra, but wild silk had a characteristic peak at 965 cm⁻¹ from the deformation vibration of the carbon-carbon double bond of the indole ring. Comparison of the pyrograms of cultivated and wild silk showed that wild silk had large indole and skatole peaks that cultivated silk did not, and these peaks might arise from tryptophan. The results of thermogravimetry/DTA showed that the endothermic peak was about 40 °C higher for wild silk than for cultivated silk. Using a combination of these results, cultivated and wild silk could be discriminated by common forensic instrumental techniques.

Keywords: Forensics, Gas Chromatography/Mass Spectrometry, Pyrolysis, Thermal Desorption
Application Code: Bioanalytical
Methodology Code: Gas Chromatography/Mass Spectrometry
Materials Science

Analytical Evaluation of Utilization Natural Cellulosic Fiber Waste as Reinforcing Filler for Rubber

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Abstract Text

Natural cellulosic fiber waste was extracted from the agricultural residues of sugarcane bagasse. It was cleaned, dried, ground and sieved to chopped micronized particles of different sizes. Some of them were chemically modified by acetylation method and stearic acid adsorption technique. The prepared fibers were incorporated into two types of rubber (NR, SBR) with vulcanizing agents through mixing process of rubber industry. The rheological characteristics were measured using Oscillating disc rheometer. The compounded rubber were vulcanized at optimum cure time. The physico-mechanical properties as tensile strength, modulus at 100% strain and elongation at break were tested before and after aging using tensile testing machine, and the obtained data were analysed. Scanning electron microscopy was applied to evaluate the homogenity and compatability of the fiber through rubber chains.

The analytical data illustrated that, the rheological and physico-mechanical behavior of rubber containing 30 phr. fiber of particle size 75 micron are good. These properties were improved and increased in case of modified fiber applied. This was confirmed by the data obtained from the swelling test and the retained values of the properties after aging. The relative toluene uptake at equilibrium swelling which define as the ratio of the weight gained after 24 hrs to the initial weight. The swelling values decrease strongly for rubber containing fiber. The adsorption of macromolecular chains at fiber/matrix interface through interactions between fiber and rubber reduce the swelling that indicate good properties of the investigated formulations. The analysis of scanning images shows good dispersion of the fiber within rubber chains matrix. So, it is recommended that 30phr chopped cellulosic fiber waste can be used as reinforcing filler for rubber after cleaning and grind to particle size range 75 -100 microns.

Keywords: Identification, Microscopy, Quality Control, Rubber
Application Code: Materials Science
Methodology Code: Physical Measurements
X-Ray Diffraction Study of Corrosion Products Formed on Anti-Weather Steel

1. INTRODUCTION
Use of weathering steel is increasing, and it became to be used to about 30% as steel materials for bridges in Japan. The life of weathering steel depends on the environments largely. The stable rust on the surface might be composed of fine $\text{FeOOH}$, $\text{FeOOH}$ and magnetite. Those materials may form protection layer on the steel. However, $\text{FeOOH}$, which is not protective rust, may be generated under the existence of chloride ion. In this study, weathering steels were corroded under the different conditions and the corrosion products were analyzed by X-ray diffraction.

2. EXPERIMENT
Corrosion test: The steel plate of COR-TEN O was cut to 80 mm x 60 mm x 1mm. Corrosion tests were carried out based on JIS H8502 method. Test solutions used are 5% NaCl, acid rain (NaCl, HNO3, H2SO4, pH3.5), acid rain “without NaCl”, 5% NaF, and 5% (NaF+Na2SO4), respectively. X-ray diffractions patterns were measured by Rigaku UltimaIV equipped with Cu target and monochromator.

3. RESULT AND DISCUSSION
X-ray diffraction patterns are shown in Fig.1. By spraying 5% NaCl solution, $\text{FeOOH}$ and $\text{FeOOH}$ were formed. Diffraction peaks of either Magnetite or $\text{Fe}_2\text{O}_3$ were observed. With respect to acid rain, $\text{FeOOH}$, $\text{Fe}_2\text{O}_3$, and Magnetite(or $\text{Fe}_2\text{O}_3$) were identified. Chloride ions promoted the formation of $\text{FeOOH}$. These data were analyzed quantitatively by Rietveld method. Detailed discussion will be presented in the poster.

References
1."COR-TEN Catalog” published by Nippon Steel

Keywords: Materials Characterization, X-ray Diffraction
Application Code: Materials Science
Methodology Code: X-ray Techniques
In this study, Polyaniline-ignimbrite (PAn-IB) which consists of electrically conductive Polyaniline (PAn) polymer and ignimbrite (IB) natural insulating material was synthesized chemically using KIO₃ as the radical initiator in aqueous media with conventional radical polymerization methods. The synthesized composites including ignimbrite with various monomer-ignimbrite percentages were monitored by scanning electron microscopy (SEM) and structural characterizations were examined using FTIR spectroscopy. Thermogravimetric analysis (TGA), particle size analysis with dynamic light scattering (DLS), magnetic susceptibility and conductivity measurements were also examined for all the synthesized composites. Making a composite with aniline, the conductivity of ignimbrite (3x10⁻⁷ Scm⁻¹), reached to 2.7x10⁻⁵ Scm⁻¹.

In the second part of the study, electrorheological (ER) behavior of the new composite material was studied. Synthesized PAn-IB nanocomposite material was granulated mechanically and the particle sizes were reduced to 20 nm level. Suspensions of PAn-IB nanocomposites were prepared in silicone oil at a series of particle sizes and their sedimentation stabilities were determined. The effects of particle sizes onto electrorheological activities of suspensions were investigated. It was observed that porous physical structure of IB material dramatically affected the ER behavior depending on the nanometer scale particle size of the composite material.

**Abstract Text**

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**Keywords:** Materials Characterization, Polymers & Plastics, Rheology, Semiconductor

**Application Code:** Materials Science

**Methodology Code:** Chemical Methods
Natural cellulosic fiber was extracted from the agricultural residues of sugarcane bagasse. It was cleaned, dried, ground and sieved to obtain chopped fiber of different particle sizes(300, 125, 75 microns). Some of them were chemically modified by acetylation method and stearic acid adsorption technique. The non and modified cellulosic fiber were incorporated into two types of rubber (NR, SBR) through mixing process of rubber industry. The rheological characteristics of rubber mixes were measured using Oscillating disc rheometer. The vulcanizates of the compounded rubber was performed at 142°C for natural rubber and 152°C for SBR. The physico-mechanical properties as tensile strength, modulus at 100% strain and elongation at break before and after aging at 90°C were tested using tensile testing machine, and the obtained data were analyzed. It was found that, the rheological and physico-mechanical properties of rubber (NR, SBR) containing different concentrations of cellulosic fiber are good after aging at 90°C. The rubber vulcanizates containing the smallest particle size at concentration 30phr showed the best retained value of properties as well as equilibrium swelling. The relative toluene uptake at equilibrium swelling defined as the ratio of the weight gained to the initial weight was measured after 24hr. The values showed that, it decrease strongly in the presence of fibers especially after treatment. The adsorption of macromolecular chains at the fiber/matrix interface occur through the interaction between fiber and rubber. The morphological analysis using scanning electron microscope (SEM) show good distribution, uniformity, and compatibility of fiber through rubber vulcanizates i.e. higher surface homogeneity and compatibility. So, it is recommended that, 30phr chopped cellulosic waste can be used as reinforcing filler for rubber after cleaning and sieving to particle size 75-100 micron.

Keywords: Identification, Materials Science, Microscopy, Rubber
Application Code: Materials Science
Methodology Code: Physical Measurements
Unconfined yield strength, measured as a function of major principle stress, is responsible for most process arches and hang-ups. However, traditional measurements of cohesive flow properties often result in poor process predictions, especially in small diameter hoppers. The major principle stress in a hopper is directly proportional to the hopper span. Thus, stress levels near small hopper outlets are very small – so small that traditional measurement techniques cannot quantify the bulk unconfined yield strength at stress levels corresponding to those expected near the hopper outlet. We postulated that the inability to predict this process behavior in small bins is because most current methods cannot measure strength values at low enough stresses to be useful. Recent advances in technology allow measurement of unconfined yield strength of fine powders at very low stress levels. To prove the technology, bulk strength values were measured using both traditional methods and the new methodology. Critical arching dimensions were computed using data from both methods and compared to direct measurements of arches in small diameter hoppers. Traditional measurement required data extrapolation to predict arching in small hoppers and resulted in arching estimates two or three times those measured in actual hoppers. Conversely, using the new method to compute critical arching dimensions resulted in predictions within 20% of actual measured arching dimensions. Thus, this new technology and test method provide prediction of process behavior significantly superior to all previous methods.
Studies have been done for years on how to analyze gas elements such as O, N and H in metallurgical materials. Traditional methods used are the inert gas fusion-infrared absorption and thermo-conductivity method. There is no standard method documented to analyze Argon or Helium in metals. Since the content of Argon in metals is very limited and the thermal conductive coefficient of Ar is too close to that of Nitrogen, traditional thermo-conductivity method can not succeed in the analysis of Ar. To solve this problem, we developed Inert Gas Fusion-Time-of-flight Mass Spectrometric Method to analyze not only O, N, and H, but also Argon and Helium. By using TOF-MS as detector, the results of O, N, H, Ar/He, etc can be gotten simultaneously. The Certified reference Materials (CRMs) of Argon/Helium are hardly to find. So a gas dosing calibration equipment was setup and has been used for calibration. Based on the calibration curve built up by gas dosing, Argon in titanium and nano powder was analyzed by using Helium as the carrier gas. The quantitative analysis results were consistent with those obtained by the modified thermal conductivity method for Ar analyzing. The differences among titanium materials were found. Argon in some specimens can be up to 700ug/g, but in the others content of Argon is too low to be detected.

Keywords: Elemental Mass Spec, Extraction, Materials Characterization, Time of Flight MS
Application Code: Materials Science
Methodology Code: Mass Spectrometry
Materials Science

Optical Properties of Aluminum Nanoparticles Experimental Determination

The research into the optical properties of metal nanoparticles is important for a number of technical applications such as cancer hyperthermia therapy, the development of solar cells etc. The task is challenging because the particles are able to scatter light as well as to absorb it. The work’s aim is the determination of metal nanoparticles light absorption and scattering parameters in a transparent medium. The experiments were carried out using pressed pellets of pentaerythritol tetranitrate containing aluminum nanoparticles (d=100 – 120 nm, 0.025 – 0.2 wt%) as experimental samples. The pellets with diameter 3 mm and variable thickness were pressed up to 10 GPa. The transmittance and the sum of transmittance and reflectance were measured with the integrating sphere. The computer programs for scattering and absorption cross sections and scattering indicatrice calculation in terms of Mie theory were developed. The illuminance calculation with spherical harmonics method assuming Fresnel boundary conditions was performed. It was shown that the reflectance becomes bigger than the Fresnel’s one. The dependence of apparent light absorption coefficient on the aluminum concentration is close to the linear one so the Beer’s law is applicable approximately. The comparison of the theoretical and experimental results at light wave length 643 nm yielded the aluminum refractive index (1.280 - 5.899i) close to the estimated by other researches. The values of apparent (1.640) and effective (0.661) absorption efficiency coefficient were determined in the experimental conditions. The work was supported by RFBR

Keywords: Light Scattering, Materials Characterization, Materials Science, Spectroscopy

Application Code: Materials Science

Methodology Code: Data Analysis and Manipulation
2-(1-morpholino)-4,6-bismaleatedethylamino-1,3,5-triazine (MBET) was prepared by reaction of 2-(1-morpholino)-4,6-bishydroxyethylamino-1,3,5-triazine and maleic anhydride. The MBET derivative was characterized by elemental analysis, acid value and spectral studies. MBET was then polycondensed respectively with three commercial epoxy resins namely diglycidyl ether of bisphenol-A (DGEBA), diglycidyl ether of bisphenol-F (DGEBF) and diglycidyl ether of bisphenol-C (DGEBC). The resultant polymers are designated as unsaturated polyester-s-triazine (UPETs) and were characterized by elemental analysis, spectral study, molecular weight determination, differential scanning calorimeter (DSC) and thermogravimetry. The interacting blends of UPETs with DGEBA epoxy resin was made at stoichiometric ratio. The blending of these systems were monitored on Differential Scanning Calorimeter (DSC) and based on DSC data the glass reinforced composites (GRCs) were prepared and characterized by physical and mechanical properties.
In the production process of paper, the elemental composition is periodically monitored and tested for the characterization of the raw and final products. Nitrogen and Carbon are the most important parameters in the quality control while the Sulfur content is an indication of impurities present in the materials. The analysis of nitrogen is required due to a specification on the nitrogen containing chemical compounds added to give the paper temperature stability. For this reason the use of an accurate instrumental analytical technique is required which avoid the use of toxic chemicals. The FLASH 2000 Analyzer permits the fast quantitative determination of the elements in paper materials without any sample pre-treatment. The system, which is based on the dynamic combustion of the sample, provides automatic and simultaneous Nitrogen, Carbon and Sulfur determination in a single analysis run. This paper presents NCS data of different paper samples multiple analyzed to show the reproducibility obtained with the system.

Keywords: Elemental Analysis, Materials Characterization, Paper/Pulp, Sulfur
Application Code: Materials Science
Methodology Code: Other (Specify)
Previously we described a novel method for clading elemental carbon onto the surface of catalytically activated silica by a chemical vapor deposition (CVD) method using hexane as the carbon source and its use as a substitute for carbon clad zirconia. In that method, a uniform monolayer of Al (III) was deposited on the silica by a process analogous to precipitation from homogeneous solution in order to preclude pore blockage. The purpose of the Al(III) monolayer layer is to activate the surface for subsequent CVD of carbon.

In this work, a more reproducible (batch-to-batch) synthesis and column packing procedure for CCSi phases was developed. A uniform monolayer of Al (III) catalyst was deposited on the surface of silica by stringently controlling the rate of the urea hydrolysis by adjusting the hydrolysis temperature and the time and amount of urea added. Removing the fine particles from the slurry before packing increased the mechanical stability of the column bed. Also, flushing the particles with a hydrogen-nitrogen gas mixture (95% N2, 5%H2) during the CVD process improved the chromatographic properties of the CCSi stationary phase without significantly changing their chromatographic selectivity. The improved chromatographic properties of the treated phase is evidenced by the decreased tailing of peaks and concomitantly in the 1.3 to 3.4 fold increase in plate counts of a carefully chosen mixture of 22 probe solutes. We believe that the improvement in peak shape results from a reduction in population of some pernicious polar sites which cause a great deal of peak tailing but which do not otherwise significantly contribute to retention.

Keywords: HPLC, HPLC Columns, Separation Sciences
Application Code: Materials Science
Methodology Code: Separation Sciences
Evaluation of Five Core Shell Columns Based on Both Separation Behavior and Physical Property

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 10 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.

In this study, a separation behavior and physical property of Kinetex C18, Accucore 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 sintering at 600 degree Celsius for 8 hours were measured. As a result, the big difference was recognized among 5 kinds of core shell C18 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.

Keywords: Liquid Chromatography
Application Code: Materials Science
Methodology Code: Liquid Chromatography
Iron(IV)-oxo porphyrins are attractive catalysts for C-H oxidation, but their reaction mechanisms are still not well-characterized due to the short lifetimes of catalytic intermediates. Recently, Zare and coworkers demonstrated that reactive desorption electrospray ionization mass spectrometry (rDESI-MS) can intercept fleeting intermediates of catalytic systems. We have combined rDESI with extractive electrospray ionization (EESI) to investigate oxidation reactions facilitated by iron(IV)-oxo porphyrins. Mechanisms of catalyst generation, catalyst degradation, and C-H oxidation were illuminated. In a typical reactive DESI experiment, one reactant is desorbed off of a surface by a DESI spray containing a second reactant, and reactions take place in the secondary microdroplets that subsequently enter the mass spectrometer. The intermediates generated in the secondary microdroplets from DESI were reacted with substrate from an EESI source, thus preventing unwanted reaction between the reagents.

Using this approach we observed formation of [Fe(III)-OOR]+ and [Fe(IV)=O]2+ species, which are key catalytic intermediates that have never been observed in previous ESI-MS studies. These experiments suggest that the mechanism of [Fe(IV)=O]2+ generation is slightly different than previously proposed in the literature; the oxidation state of the porphyrin ring may be decoupled from O-O bond cleavage, rather than being oxidized only from O-O bond heterolysis. In addition, numerous intermediates in a bimolecular degradation pathway indicate that rapid degradation of the porphyrin ring (via loss of a meso-phenyl substituent) accounts for the loss of catalytic activity. To verify catalytic reactivity, rapid oxidation of propranolol by the in situ-generated [Fe(IV)=O]2+ was carried out using the combined rDESI-EESI method.
Nowadays, LA-ICP-MS represents a powerful tool for determination of minor and trace elements in geological samples. However, the problem appears if some elemental map of heterogeneous geological samples should be created. The problem consists in two facts. The first one, the ablation rate of each phase of the sample differs. This problem could be easily solved using internal standard. However, the second problem arises now. We analyze heterogeneous samples hence each phase can have different internal standard and its content may also vary.

In presented work we describe the possibility of elemental mapping heterogeneous geological samples using LA-ICP-MS. For this purpose we used setup consists of laser ablation system UP213 (NewWave, USA) operating at wavelength of 213 nm and quadrupole ICP-MS Agilent 7500ce (Agilent, Japan). The presented elemental maps were obtained by laser ablation of granitoids from Bohemian massif what represents sufficient heterogeneous samples (mica, quartz, feldspar ...). The new lab-made software was used for creation of elemental maps.

Authors gratefully thank for financial support from project CEITEC CZ.1.05/1.1.00/02.0068.
Development of the marine resources and protection of the maritime environment are the current trend in the world. For those, chemical composition of materials should be evaluated firstly, so analytical technology is essential to the fundamental studies. The main objective of this study is to determine 18 kinds of elements in marine sediments of the manganese crusts (China standard reference materials of GBW07296 and GSMC-1) by ICP-AES, which uses the high pressure digestion tank method for sample preparation. The pretreatment procedure of samples, the optimum analytical lines of the determined elements, the operational parameters and the influence of coexisting elements for inter-element correction were investigated. The results showed that the quantitation values matched the certified value for those standard reference materials. The %RSDs for determination of all elements were better than 2.05%. This method is rapid and convenient with high accuracy and good precision, which is suitable for the ocean exploration and research.
In spite of the worldwide boom in traditional Chinese herbal medicine (CHM) in recent years, its associated health risks for users due to arsenic and heavy metal contamination, has led to growing public concerns. Arsenic speciation in soils, the effect of arsenic on the growth of various CHM as well as the uptake and accumulation of arsenic in different part of the plant was investigated. Arsenic concentrations in fresh plant samples can be very low which poses a great challenge for the speciation analysis. A two-step pre-concentration procedure was proposed in this presentation. After extraction, DMA, MMA and arsenate in the extract was collected using an anion exchange cartridge. The residue of the extract was then acidified (pH 2) followed by the addition of APDC. At this condition, arsenite forms a complex with APDC which was then trapped using a C18 cartridge. Arsenic species on both cartridges were then eluted using methanol followed by the analysis using LC-HG-AFS. The proposed procedure not only enables the speciation of arsenic at very low concentration, also greatly reduced the risk of species transformation after the extraction.
### Session Title
Trace Metals and Gasses by AA, ICPMS, ICAFS

### Abstract Title
**Preliminary Results for Metals Found in Venison from White-Tailed Deer from Northwestern Pennsylvania**

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### Abstract Text
This presentation will focus on determination of selected metal analytes (Fe, Zn, Cu, Mn, Pb) in venison from white-tailed deer harvested from Northwestern Pennsylvania. The purpose of this research endeavor is to assess the extent (if any) of potential environmental contamination, especially toxic metals such as Pb, As, Cd, and Hg. Three separate venison samples were collected from each deer and microwave-digested and then analyzed for the selected metals by flame atomic absorption spectrometry (FAAS). Sample preparation and analytical determination protocols, along with future plans for this work, will be presented and discussed.

### Keywords:
- Atomic Absorption
- Environmental/Biological Samples
- Metals
- Sample Preparation

### Application Code:
Environmental

### Methodology Code:
Atomic Spectroscopy/Elemental Analysis
This presentation will focus on preliminary studies to assess the ability of palm plants to accumulate arsenic from contaminated soils. Prior research by our group on phytoremediation of arsenic was performed with sunflowers, which are well known for their ability to hyperaccumulate arsenic. Tomatoes and certain types of ferns are also known for their ability to hyperaccumulate arsenic from contaminated soils. A prior attempt in our group to assess arsenic uptake by lettuce yielded inconclusive results. There appears to be very little if any available literature on accumulation of arsenic using palm plants; thus, the possibility of arsenic uptake by palm plants was considered by the authors and explored.

Three sets of palm plants were used in this study: a control set unexposed to arsenic, and two sets watered with 2.0 mg As/L and 10.0 mg As/L standard solution, respectively. The method used for determination of arsenic was hydride generation atomic absorption spectrometry (HGAAS) preceded by microwave digestion of selected portions of the palms.

Experimental protocols, such as plant growth and watering with arsenic-containing solution of known concentration, sample preparation techniques, and the determination of arsenic in the palm plant samples by HGAAS, will be presented and discussed, as will results of the arsenic determinations calibration data for the HGAAS method, and future directions for this research.

Keywords: Atomic Absorption, Environmental, Hydride, Sample Preparation

Application Code: Environmental

Methodology Code: Atomic Spectroscopy/Elemental Analysis
Trace Metals and Gasses by AA, ICPMS, ICAFS

A New Modular Approach to Automated Cold Vapour and Hydride Generation AFS for Mercury and Hydride Forming Elements

PS Analytical have been manufacturing fully automated cold vapour and hydride generation AFS systems for over thirty years. The determination of mercury and hydride forming elements such as As, Se and Sb continues to be a challenging task for many laboratories because of the low levels often encountered. The cold vapour and hydride generation techniques require sample preparation beyond digestion to ensure good hydride and Hg vapour generation yields. This is because Mercury and the hydride forming elements may occur as organic metallic species and also in different valence states even after sample dissolution. The problem is not unique to vapour generation techniques as the volatility of species may also affect the sample introduction efficiency of ICP systems. Sample preparation is typically done offline prior to measurement which can be time consuming and in some cases detailed knowledge of the species present in the digest is needed. When coupled to liquid chromatography to offer speciation, there is a need to perform post column conversion of eluted species prior to the vapour generation step.

In this paper we will introduce a new series of modular products based on vapour generation AFS for mercury and the hydride forming elements. One of the modules that will be discussed provides automated sample preparation by use of an UV photolysis system that can operate at elevated temperatures. The system has a built in LC pump and injection valve to provide a compact versatile speciation module. The benefits of this new approach will be discussed with supporting analytical performance data for a new range of products.

Keywords: Atomic Spectroscopy, Hydride, Mercury, Speciation
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Trace Metals and Gasses by AA, ICPMS, ICAFS

Identification and Characterization of Heavy Metal of Baby Powder Using Laser Induced Breakdown Spectroscopy (LIBS)

Nowadays, there is growing interest for scientists to develop analytic techniques which could give fast and reliable results without much destruction of the sample. For that purpose a versatile technique - Laser induced breakdown spectroscopy (LIBS) is gaining interest. Laser induced breakdown spectroscopy (LIBS) is an Atomic Emission Spectroscopy (AES) that is widely used in various fields such as material science, forensic science, biological science and chemical and pharmaceutical industries. Here we apply it to various baby samples which we know is derived from talcum powder. Talcum powder can pose considerable threats if inhaled in larger excess, accidentally. Talc is the most deleterious ingredient in talcum powder. Inhalation of talc by babies might lead to choking and asphyxiation. This suggests potential presence of toxic heavy metals. The goal of this study is to develop spectral signatures in order to distinguish various types of baby powder available in market and also their quality in term of toxic element present in the powder. In this study we have collected LIBS spectra by varying various parameters such as laser energy, gate delay and gate width to optimize the LIBS signals. Qualitative analysis was performed in order to identify heavy metals such as Pb, Cr, Cd, Si, Mg, Al, Ti, Fe, Mn, Ca. Elemental ratios in various powder were then calculated in order to develop a sample signature.

Keywords: Analysis, Atomic Emission Spectroscopy, Atomic Spectroscopy, Characterization
Application Code: Other (Specify)
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Recently, it has been shown that isotopic information from a sample can be obtained via laser-induced breakdown spectroscopy (LIBS) in a technique known as laser-ablation molecular isotopic spectroscopy (LAMIS). LAMIS involves the analysis of diatomic molecules that are produced after atomic recombination of isotope-enriched materials in a laser-induced plasma. When elemental breakdown occurs, diatomic isotopologues are formed that exhibit a wavelength shift in their optical emission spectra. These shifts arise from the differences in the vibrational and rotational energies of the isotopologues and facilitate the determination of the relative abundance of isotopes in the material for LIBS analysis. The original LAMIS work has focused on single-pulse (SP) LIBS for the excitation. However, dual-pulse (DP) LIBS reduces shot-to-shot variation and can lower detection limits of an element by about an order of magnitude or more. It also has the potential to improve the accuracy of the determination of the relative abundances of isotopes in LAMIS by minimizing the signal-to-noise ratio.

In this work, a DP-LIBS technique for improving LAMIS relative-abundance information from a sample is presented. The new technique, called (TEA) Transverse-Excited breakdown in Atmosphere Laser-Enhanced Laser Ablation Molecular Isotopic Spectrometry (TELLAMIS), uses a carbon dioxide (CO\textsubscript{2}) laser to increase the breakdown emission from LIBS in the LAMIS method. This technique is demonstrated on a collection of relative abundance isotopes of boron-10 and boron-11 in varying concentrations in boric acid. Least-squares fitting to theoretical models are used to deduce plasma parameters and understand reproducibility of results.

**Keywords:** Atomic Emission Spectroscopy, Chemometrics, Nuclear Analytical Applications, Plasma Emission (ICP/MS), Nuclear

**Application Code:** Atomic Spectroscopy/Elemental Analysis
Increase in industrial activity and world population has resulted in soil heavy metal environmental contamination and pollution in recent years. Heavy metal pollution is a topic of considerable health and environmental concern because many health issues including cellular damage, organ failure, cancers, depression, and developmental problems in children have been associated with elevated concentrations of lead, cadmium, mercury, and chromium. Humans can be potentially exposed to heavy metals through occupational and environmental exposure, consumption of heavy metal contaminated foods, and industrial pollution, among others. The potential deleterious effect of heavy metals necessitates an immediate remediation strategy to clean the contaminated sites. Consequently, heavy metal remediation techniques such as phytoremediation, the use of organic acids, chelating agents, modified adsorbents, and surfactants have been widely employed for heavy metal remediation. While these methods do provide some success, they have some disadvantages ranging from long remediation time, poor remediation efficiency, poor metal selectivity, high costs, disposal of remediation waste, and mobilization of these metals. There is therefore the need for the development of a non-toxic, more efficient, and environmentally friendly method for the remediation of heavy metals. The findings from this study showed that remediation efficiency is heavy metal dependent and also depends on the molecular micelles used for kaolin clay modification. Also, the pH of the solution and the remediation time has a significant effect on the remediation efficiency of the kaolin adsorbents. Generally, all investigated kaolin adsorbents provided relatively good remediation efficiency of all heavy metals, with remediation efficiencies ranging from 55% and 90%.

Keywords: Absorption, Atomic Absorption, Atomic Spectroscopy, Environmental/Soils
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Carbon capture and storage (CCS) has been proposed as a viable means for reducing anthropogenic carbon dioxide (CO$_2$) emissions. This entails the injection of supercritical CO$_2$ in deep underground brine-filled reservoirs, which requires the CO$_2$ to remain either supercritical, or in solution in the saline formations. However, risks of leakage of the injected CO$_2$ or resident fluids such as brine, is a major concern associated with the injection of large volumes of CO$_2$ in storage formations. For instance, migration of CO$_2$-laden brine could contaminate drinking water resources, or endanger vegetation and animal life as well as human health.

We propose the use of laser-induced breakdown spectroscopy (LIBS) technique for evaluating potential leaks of CO$_2$-laden brine from the injection sites. For comparison, LIBS has been performed in aqueous solution containing either dissolved carbon dioxide or nitrogen gas to study calcium emission signal. A microplasma was formed in bulk CaCl$_2$ solution by focusing a Nd:YAG laser beam operating at 1064 nm through Sapphire window of a high pressure vessel. Results demonstrated that calcium emission exhibits a faster temporal decay in presence of dissolved CO$_2$ or N$_2$ than measurements conducted at ambient conditions. The dependence of Ca emission on dissolved gas was studied by performing the experiment at different pressures from 10 to 120 bar. Electron densities calculated from Stark broadening of Ca I 422.67 nm line and plasma temperatures calculated from the intensity ratio of Ca II 393.4 nm to Ca I 422.7 nm lines were measured in the three gases pressures.
Trace Metals and Gasses by AA, ICPMS, ICAFS

Application of ICP-MS in Assessing the Abundance of Rare Earth Elements (REE) in Marcellus Shale Cores

Many shale deposits are known to accumulate rare earth elements (REEs). REEs in shale deposits are receiving increased attention because of their predictable geochemical characteristics and potential economic value. For unconventional gas extraction processes, REEs show potential as naturally occurring tracers of subsurface fluid migration both during and after hydraulic fracturing as well as tools for source identification and apportionment in the event of shallow groundwater contamination. In this study, we screened Marcellus Shale outcrop samples for REEs using inductively-coupled plasma mass spectrometry (ICP-MS). These samples were collected from different organic-rich shale formations from seven geographic locations in West Virginia, Virginia, Pennsylvania, and New York with surface exposures of the Middle Devonian Marcellus. The sample digestion employed HF/HNO3 and fusion techniques to assess quantitative recovery of REEs. Analysis was performed using an external calibration technique and internal standards were used to correct for instrumental drift and matrix effects. The REEs tend to fractionate during diagenetic processes and are shown to be associated with various mineral facies, e.g. the organic fraction in shales. Understanding of geologic abundance and variability is critical to future applications of REEs as tracers in the aqueous phases in contact with these shales.

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Atomic Spectroscopy, Environmental, ICP-MS, Trace Analysis

Environmental

Atomic Spectroscopy/Elemental Analysis
Trace levels of Selenium are present in coal. With tightening regulations on acid gas emissions from coal fired utilities there is an increasing trend to install flue gas desulfurization (FGD) systems. Wet scrubbers based on limestone forced oxidation remove a portion of the Selenium and other volatile metals. The FGD waste water may include particulate and various dissolved forms of Selenium. With regulatory discharge levels typically set to below 10 part per billion (ppb) there is a need to treat FGD wastewater to remove Selenium and also to confirm the effectiveness of the removal process by speciation analysis. Total selenium in FGD wastewater was analysed using HG-AFS followed by the online UV digestion at elevated temperature. The samples were also analysed for total selenium using ICP-MS for comparison. Selenium species in the wastewater was analysed using HPLC-HG-AFS as well as IC-HG-AFS. Under HPLC separation mode, a citric buffer (pH 5) mobile phase was used which was able to recover selenite and selenate in the sample, while the majority of the selenium in the sample was unable to elute from the column. Samples were also analysed using IC-HG-AFS under gradient alkaline elution. Various selenium species including Selenocyanide (SeCN) were found using this method which account for the majority of the total selenium concentration in FGD wastewater.

Keywords: Atomic Spectroscopy, Clinical/Toxicology, Mercury, Sampling
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The first step in the pyrometallurgical process for extracting copper and nickel from ores consists in melting the ore and skimming the silicates and other oxides that float on the surface. The denser mixture of molten metals and sulfur is found at the bottom is called the matte. Our industrial partners want to know the concentration of copper, nickel and cobalt in the matte, in real time and in situ in the furnace or immediately at the exit of the furnace when it is tapped. Knowing these concentrations would allow increasing production efficiency in the following processing step, whereby iron and sulfur are oxidized inside a second furnace called the converter.

We are developing a laser-induced breakdown spectroscopy (LIBS) sensor to measure online the concentrations of copper, nickel, cobalt, iron and sulfur in the molten matte. The measurement is made through a tube in which an inert gas flows. This allows measuring below the surface slag and impurities. Alternately, we are also investigating direct measurements onto the surface which could be advantageous when the molten matte is flowing in a channel. The advantages of LIBS technology are speed, lower operation costs than the current sampling technique, and improved safety because LIBS does not require manual sampling of molten metals. Preparatory measurements taken in the laboratory at temperatures of approximately 1100 [degree]C will be presented. From an analytical point of view, one difficulty is that there is no major element that can be used to normalize line intensities.

Keywords: Atomic Emission Spectroscopy, Plasma Emission (ICP/MIP/DCP/etc.), Process Control, Sensors
Application Code: Process Analytical Chemistry
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Fingernail and hair samples are reliable indices of metal concentrations in the human body. However, the methodology utilized to prepare these samples can cause significant variability in the experimental results. In some cases, published concentrations of a given metal in the same matrix vary by up to two orders of magnitude. Given the variability in published results and our own preliminary analyses, the objective of this study was to characterize the accuracy and precision of data obtained using two sample preparation methods for both fingernail and hair samples. Samples were collected from healthy adults, cleaned and digested, and analyzed for strontium, selenium, zinc, lead, mercury, cadmium, and iron using inductively coupled plasma-mass spectrometry (ICP-MS). Results were processed using chemometric methods to determine if there were statistically significant differences between fingernail or hair matrix results and to explore the impact that variables such as environmental toxin exposure, diet, gender, and age have on the data. Future research will explore relationships among metal concentrations in nail and hair samples and environmental health, specifically environmental tobacco smoke exposure.

Keywords: Chemometrics, ICP-MS, Metals, Trace Analysis
Application Code: Environmental
Methodology Code: Mass Spectrometry
Using plants as bioindicators has become popular in recent years. With the increasing use of heavy metals in industry and the subsequent disposal in landfills, it has become important that the mutagenic effects of heavy metal contamination be studied. Nickel salts are thought to be carcinogenic to humans and lab animals; however their effects on plants are far less studied. Nickel(II) chloride was chosen because of its wide use in chemical industry, which has led to higher runoff concentrations in soil and water. This study focuses on the physiological and mutagenic effects of Nickel(II) chloride on the Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene in \textit{Arabidopsis thaliana}.

Plants were grown in soil containing various concentrations of Nickel(II) chloride, as well as uncontaminated soil to serve as a control. Nickel content of the soil and plants will be determined according to EPA method # 3050B. The plants' DNA will then be extracted and the GAPDH gene amplified, purified and sequenced. The control and nickel-treated DNA sequences will then be analyzed and compared for any changes in base pair sequence. Flame Atomic Absorption Spectroscopy and/or inductively coupled plasma mass spectrometry will be utilized to determine nickel concentration in the soil and plants. Correlations between nickel concentrations and physiological changes or mutagenic effects on the GAPDH gene will be presented.

**Keywords:** Agricultural, Atomic Absorption, Atomic Spectroscopy, Soil

**Application Code:** Bioanalytical

**Methodology Code:** Atomic Spectroscopy/Elemental Analysis
Laser ablation ICP-MS is becoming increasingly popular in the field of elemental imaging. It is very versatile, useful for various sample types, with a wide dynamic range and low detection limits. We present a development of a 2D LA-ICP-MS imaging procedure for ancient glass that successfully extracts the sample's surface elemental features. The laser beam is scanned across the surface of the sample along parallel lines and the data obtained are manipulated so that a "map" of the element distributions is obtained. Using a sum-normalization calibration strategy\[1\], element distributions are converted to quantitative 2D elemental images. Maps for 54 elements (oxides) in archaeological glass\[2\] were obtained this way (Fig. 1). To further investigate processes such as weathering or degradation of glass surface layers, a novel 3D LA-ICP-MS analysis approach was developed recently\[3\]. This approach is a combination of depth profiling and rastering, capable of direct 3D mapping of "hard" materials such as glass. The 3D imaging procedure (see Fig. 2) features slow laser drilling on a grid on the sample surface and ultrafast ICP-MS acquisition to resolve individual ablation pulses. Acquired data is manipulated so that 3D quantitative elemental (volume) images are created. We successfully tested this approach to investigate mechanisms behind the degradation of a medieval, weathered glass artifact using colocalization analysis of selected cross-sectional 2D elemental images constructed in arbitrary planes of the acquired 3D volume images.

References:

Keywords: Art/Archaeology, ICP-MS, Imaging, Laser
Application Code: Art/Archaeology
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Image analysis can be a powerful tool in defect/failure analysis in Axalta Coating Systems’ automotive applications. These tools can identify size, shape and populations of defects, as well as extent of damage, locus of failure and material types. Numerous applications have been explored over the last ~5 years, including (1) particle size/shape tracking, (2) grit/dirt/fiber contaminant studies, (3) blister formation tracking, (4) cross-hatch extent of damage and locus of adhesion failure, (5) Gravelometer damage testing, (6) crater or pin-hole defect studies, (7) dispersed particle/aggregate analysis, and (8) substrate corrosion analysis. This presentation will highlight a number of Axalta Coating System relevant applications and provide a detailed review of the entire image processing sequence. The work detailed herein was undertaken as bootleg to demonstrate the broad utility of the ImageJ system.

ImageJ is effectively free software which has been developed in Java. As a result it can run under various operating systems or platforms, including Windows, Mac OS X and Linux. It is capable of working with a broad range of common image types, including the JPEG, BMP, GIF and TIFF. Also, Macros can be easily written or downloaded to automate a variety of tasks. Note - many Macros are now available on the ImageJ website.

**Abstract Text**

Image analysis can be a powerful tool in defect/failure analysis in Axalta Coating Systems’ automotive applications. These tools can identify size, shape and populations of defects, as well as extent of damage, locus of failure and material types. Numerous applications have been explored over the last ~5 years, including (1) particle size/shape tracking, (2) grit/dirt/fiber contaminant studies, (3) blister formation tracking, (4) cross-hatch extent of damage and locus of adhesion failure, (5) Gravelometer damage testing, (6) crater or pin-hole defect studies, (7) dispersed particle/aggregate analysis, and (8) substrate corrosion analysis. This presentation will highlight a number of Axalta Coating System relevant applications and provide a detailed review of the entire image processing sequence. The work detailed herein was undertaken as bootleg to demonstrate the broad utility of the ImageJ system.

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**Keywords:** Data Analysis, Imaging, Microscopy, Surface Analysis

**Application Code:** Polymers and Plastics

**Methodology Code:** Other (Specify)
Phosphating is used for metal passivation. It improves the corrosion resistance of coated surfaces. Prior to the phosphating process, several steps are required such as: Degreasing using a hot alkaline solution to remove dirt, oil, grease, shop oil and soluble markings; Pickling using dilute solutions of either hydrochloric or sulfuric acid to remove surface rust and mill scale to provide a chemically clean metallic surface; Fluxing using a salt solution to remove oxides and to prevent oxidation. Monitoring the level of elements in baths is critical to ensure an efficient process. Both major elements and trace contaminants have to be monitored and ICP-OES is an excellent tool due to its capability to analyze major and trace elements with a multi-element analysis capability and a reduced sample preparation time.

Results obtained on a large variety of samples are presented in this poster. Analysis was done on a radial viewing based ICP-OES with Total Plasma View feature allowing the measurement of the whole Normal Analytical Zone of the plasma for improved sensitivity. The Far UV capability allows Chlorine analysis, avoiding the need to analyze the sample with other techniques and thus improving laboratory throughput.
Trace Metals and Gasses by AA, ICPMS, ICAFS

High Salt Content Samples Analysis Using Radial Viewing ICP-OES Instrument with Total Plasma View Feature

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 Emission Spectroscopy, Elemental Analysis, ICP
Application Code: General Interest
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Traceability of gas-phase mercury analyser calibration is of particular concern with all mercury analysis. It is important to ensure that the calibration used is appropriate for the sample being analysed, particularly in terms of the species present and, in some cases, the sample matrix. Elemental mercury calibration is widely available. In some cases, such as ambient air and coal-fired utility stack monitoring, oxidised sources of mercury are needed for calibration and system integrity tests. Calibration of mercury analysers used for the automated monitoring of ambient air is frequently in the 0.1-10 ng mass range. This can be orders of magnitude above the masses collected during air monitoring, with the results measured close to the calibration zero-points, with resulting errors. Alternative calibration techniques and analyser operations can be adopted to ensure the results are interpolated on the calibration curve. Suitable calibration strategies can be particularly difficult where transient emissions are monitored, for example waste incinerators or crematoria. Here a very wide dynamic range is required, with ‘baseline’ emissions at sub-µg/m³ levels and transients at mg/m³ to saturated levels. To ensure that average emissions are measured accurately, good calibration is required in both of these ranges. (Inaccurate ‘baseline’ emission results over hours or days can affect the average emissions levels as much as than poorly monitored mg/m³ spikes.) When used in industrial settings, it is important that analyser calibrations are straightforward and readily understood by the equipment operators. Analytical considerations can easily be misunderstood or neglected in favour of user convenience, particularly as users are unlikely to have an analytical or scientific background. This poster will provide an overview of analytical challenges for the calibration of gas-phase mercury analysers, presenting some examples and solutions.

Keywords: Atomic Spectroscopy, Calibration, Mercury, Monitoring
Application Code: Regulatory
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Trace Metals and Gasses by AA, ICPMS, ICAFS

Mercury Release Rates from Dental Amalgam: Measurement and Sampling

The use of mercury amalgam fillings in modern dentistry continues to cause controversy. Amalgam fillings are accepted practice in much of the world whilst their use is restricted in others. Restrictions on the use of amalgam fillings have been advocated on health grounds – the mercury exposure to patients and those preparing, fitting and/or removing being relevant – and on environmental grounds. One consideration appropriate to this debate is the mercury release rate from amalgam fillings. The release rate from a dental amalgam surface may be affected by a wide range of factors, including surface area, temperature, consumption of foods and drinks (temperature and chewing) and bruxism.

This poster presents a simple method for measuring the mercury release rate from dental amalgam using amalgamation-atomic fluorescence spectrometry. Sampling strategies are discussed and data presented showing optimisation of flow rate and sample monitoring. Mercury release rate data are presented from a self-selected sample of approximately 70 persons.

Keywords: Atomic Spectroscopy, Clinical/Toxicology, Mercury, Sampling
Application Code: Clinical/Toxicology
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The Fukushima Daiichi nuclear disaster is a series of nuclear meltdowns accident and releases of radioactive materials from the Fukushima-1 Nuclear Power Plant, following the Tohoku earthquake and tsunami on 11 March 20 in Eastern Japan. For the accident, isotope ratio analysis for $^{235}\text{U}$ and $^{238}\text{U}$ in soil sample using a microwave digestion procedure associated with ICP-MS has been developed. At the microwave digestion procedure, the dissolution of the natural uranium in silicate (in rocks) was reduced by the use of mixture of nitric acid and hydrogen peroxide (to measure the radioactive uranium from disaster). On ICP-MS, the isotope ratio of the certificated geochemical reference material was realized to precisely correct the isotope ratio in real soil sample. In addition, cell-pass voltages can be available to make calibration and/or to correct the mass bias in mass-spectrometer. By these effects, the isotope ration of uranium can be measured at accuracy of 0.37% without the use of the radioactive standard source. $^{235}\text{U}$ and $^{238}\text{U}$ were quantitatively determined and those detection limits were both 0.010 $\mu$g/kg. In the case of emergency like a nuclear hazard, proposed method is useful to immediately gather large areal information as compared with the common method such as a complete dissolution process associated with X-ray spectrometer or ICP-MS. In addition, the survey of the radioactive uranium spreads was conducted from 5 to 80 km around the Fukushima Daiichi Nuclear Power Plant (Fukushima-DNPP)(115-points in Fukushima prefecture). As the results, the values of uranium isotope ratio for those soils were similar to the natural abundance, although the various concentration of uranium was detected from sampling points.
Stability, Linearity and Repeatability of Nitrogen Determination by Flash Combustion Using Argon as Carrier Gas

Elemental analyzer with a thermal conductivity detector for Nitrogen 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 it 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 content.

This paper presents data on Nitrogen 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, Method Development
Application Code: General Interest
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Trace Metals and Gasses by AA, ICPMS, ICAFS

Fast PDMS Quantitation Using ICP-OES

The reliable quantitation of polydimethylsiloxanes (PDMS) in aqueous media is made challenging by their ubiquitous presence in the environment and their ability to “stick” to surfaces. Herein we present a method developed for quantifying Antifoam C (30 wt % PDMS) in aqueous solutions, by inductively coupled plasma-optical emission spectroscopy (ICP-OES), using a Thermo iCAP 6300 Duo. Our method, which determines the PDMS content from the total silicon, is based on a simple extraction in organic solvents and produced good recoveries for both high and low silicon contents. With this method we proved that PDMS can be measured reliably and with minimal sample preparation (i.e. avoiding acid digestion) via total silicon content.

Keywords: Atomic Emission Spectroscopy, Elemental Analysis, ICP, Trace Analysis
Application Code: Polymers and Plastics
Methodology Code: Atomic Spectroscopy/Elemental Analysis
The Trent and Mersey Canal is located in Middlewich and has been subject to historic pollution for a number of years. A chemical plant by the name of Hays which produced inorganic chemicals used to discharge treated waste effluent contaminated with mercury into the Canal. The discharge points have been detected at 3 different sites over the years. Pre 1975 the discharge point was located by the bridge opposite the site. From 1975 to 1990 the discharge point was then by Crows Nest Lagoons, and from 1990 to 2010 the discharge point was identified 1km upstream from Crows Nest Lagoons. 9 cores were taken covering a 9km stretch of the Canal and then were analysed for total and methyl mercury using a PSA atomic fluorescences spectrometry attached to a Millennium Merlin spectrometer. The concentration of total mercury was between 1.56 $\mu$g/g and 625.81 $\mu$g/g. The concentration for methylmercury ranged from 0.86ng/g to 51.78ng/g. The Central sites seemed to be more contaminated than the upstream sites ($P=0.01$). This may be due to changes in discharge consent levels over the years, coupled with canal velocity and boat movement transporting upstream plume downstream. Loss on Ignition was performed to see if there is a correlation between organic matter and total mercury, there was no correlation ($R^2= 7E-06$). However, there was a correlation between organic matter and methylmercury ($R^2=0.51$). There was also a relationship between total and methyl mercury ($R^2=0.62$). The total mercury concentrations detected in the Canal have exceeded both the consensus-based effect range medium (0.17 $\mu$g/g) and the probable effect concentration (1.06 $\mu$g/g). The methylmercury levels constitute less than <1% of total mercury levels and this is considered to be the desirable range in sediments.
Seasonal variations in Water quality parameters such as Water Temperature, Water Transparency, pH, TDS (Total dissolved salts), Total Hardness, Calcium Hardness, Magnesium Hardness, Chloride (Cl), BOD (Biological oxygen demand), COD (Chemical oxygen demand), DO (Dissolved oxygen), Alkalinity were analysed twice in a month for a period of two years during 2010-2011 and 2011-2012. All parameters were within the permissible limits of Indian Water Quality Standard. The result indicates that Wetlands of Kheda district are not polluted and the water can be used for irrigation, pisciculture and Domestic purpose.

Keywords: Water
Application Code: Environmental
Methodology Code: Chemical Methods
Studies of Physico-chemical analysis such as Temperature, pH, dissolved oxygen (DO), total dissolved solids (TDS), electric conductivity (EC), total alkalinity (TA), calcium hardness, magnesium hardness, chloride (Cl), sulphate, nitrate of water samples of bore wells of twenty villages of Kheda district, Gujarat, India. Quality of water is an important factor for drinking. Some villages were found to have magnesium limit and minimum tolerance range for drinking water. The experimental values of water samples were compared with standard values given by world health organisation (WHO) and Indian standards. The results shows that quality of water is poor and quite good for drinking and irrigation purposes respectively, In the present communication deals with study of phosphate parameters is higher than the prescribed values. The higher values of phosphate are mainly due to use of fertilizers and pesticides by the people residing in this area. If the phosphate is consumed in excess phosphine gas is produced in gastro-intestinal tract on reaction with gastric juice, Similarly Nitrates 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 bore well water. All these parameters of bore wells water were carried out during 2011-2012 in order to water quality index.

Keywords: Water
Application Code: Environmental
Methodology Code: Chemical Methods
Water Quality Parameters: Still Providing Important Information

Rapid Determination of Ultimate Biochemical Oxygen Demand (Ultimate BOD)

The BOD5 is defined as the amount of dissolved oxygen consumed by bacteria as they oxidize readily decomposable organic material under very specific conditions during a 5-day test. This 5 day test is required by regulation, but does not necessarily provide an accurate assessment of the total concentration of organic material leading to an underestimation of the true oxygen demand of a waste stream. Most ultimate BOD tests carry the 5-day BOD to completion, sometimes taking up to 20 days before a reliable result is available. This poster describes a rapid, low cost method for BOD that rapidly oxidizes all bioavailable organic material present in a sample reporting the result as oxygen demand.

Abstract Text

Keywords: Analysis, Environmental, Environmental Analysis, Environmental/Water
Application Code: Environmental
Methodology Code: Chemical Methods
Total Kjeldahl Nitrogen (TKN) is the USEPA-approved parameter used to measure organic nitrogen and ammonia. The TKN content of influent municipal wastewater is typically between 35 and 60 mg/L. Organic nitrogen compounds in wastewater undergo microbial conversion to ammonia and ammonium ion. Ammonium is removed from wastewater by nitrification, a two-step biological process that converts ammonium to nitrite and the nitrite to nitrate. A denitrification process reduces nitrate generated in the preceding steps to nitrogen gas.

U.S. EPA methods 351.2 for TKN and 350.1 for ammonia have traditionally involved a manual distillation step prior to analysis. Distilling samples is time consuming, reduces sample throughput, and increases laboratory costs for labor, reagents, and consumables. In-line gas diffusion is an approved method modification specifically cited by the U.S. EPA for TKN and ammonia analyses in the Final Rule published in the Federal Register of May 12, 2012.

Keywords: Automation, Environmental/Water, Spectrophotometry, Wet Chemical Methods
Application Code: Environmental
Methodology Code: Chemical Methods
This investigation will focus on the determination of selected metals (Al, Fe, Mn Ca, Zn) and nonmetals (P, N, dissolved oxygen) in both clean and contaminated bodies of water in western Pennsylvania and western New York. The purpose of this investigation is to examine the potential effects of industrial, residential, and agricultural runoff from the areas surrounding three diverse natural water sources (Parry Lake, western NY; Lake Erie, PA; and abandoned mine drainage settling ponds, Lowber, PA). Water samples collected will be acidified with dilute nitric acid (HNO₃) to preserve the metals present, filtered if needed, then analyzed for the aforementioned analytes. Phosphorus and nitrogen will be determined by visible spectrophotometry. The metals (including calcium) will be determined using flame atomic absorption spectrometry (FAAS). Dissolved oxygen and calcium will be determined on-site at Lowber by ion-selective electrodes. Additionally, pH, conductivity and water temperature will be measured on-site. Sample preparation and analytical determination protocols, along with the results obtained from this research, will be presented and discussed.

Keywords: Atomic Absorption, Environmental/Water, Ion Selective Electrodes, Metals
Application Code: Environmental
Methodology Code: Atomic Spectroscopy/Elemental Analysis
Wastewater treatment plants (WWTPs) use the Biological Oxygen Demand (BOD) test to measure oxygen consumed by decomposition of organic matter in secondary wastewater treatment processes. Bacteria, algae, fungi and other microorganisms consume and remove up to 90 percent of organic matter during secondary wastewater treatment. Total organic carbon (TOC) analysis provides a direct quantitative measurement of organic contamination in water and wastewater, whereas BOD tests provide an indirect, empirical estimation of organic contamination.

In 40 CFR 133.104 the U.S. EPA allows wastewater treatment plants to substitute TOC analysis for BOD monitoring of oxygen-demanding substances. WWTPs seeking to substitute and report TOC values for BOD values must conduct a long term correlation study and submit the results to the regulatory body that issued the NPDES permit for their facility. Study data must be collected using USEPA-approved methods intended for NPDES permit compliance reporting.

This poster will present comparative data obtained on wastewater samples using laboratory and on-line TOC analyzers employing the heated sodium persulfate oxidation technique in USEPA-approved method SM 5310C.
Current research deals with a variety of nanoparticles (NPs) of widely varying composition (e.g., metals, semiconductors, organics, and composites) and structure (spheres, cubes, wires). While microscopic and spectroscopic methods are widely used, all suffer from limitations. However over the past years electrochemical methods, based on observation of single NP collisions with an electrode have been developed. These are based on using the electrochemical response and the frequency of collisions to extract information about the NPs. An overview of this field will be given and recent advances described.
Amperometric currents displaying a pre-spike feature (PSF) may be treated so as to lead to precise information about initial fusion pores, viz., about the crucial event initiating neurotransmitters vesicular release in neurons and medullary glands. Since amperometric data alone are not self-sufficient their full exploitation requires an external calibration for solving the inverse problem. For this purpose we resorted to patch-clamp measurements published in the literature on chromaffin cells. Reported pore radii were thus used to evaluate the diffusion rate of neurotransmitter cations in the partially altered matrix located near the fusion pore entrance. This allowed an independent determination of each initial fusion pore radius giving rise to a single PSF event. The statistical distribution of the radii thus obtained provided for the first time an experimental access to of the potential energy well governing the thermodynamics of such systems.

The shape of the corresponding potential energy well strongly suggested that, after their creation, initial fusion pores are essentially controlled by usual physicochemical laws describing pores formed in bilayer lipidic biological membranes, i.e., have an essential lipidic nature.

Based on the established rate of diffusion inside the vesicle, the dimensions and energetics of the time dependent fusion pore radius can be extracted from each recorded main spike current. This leads to the conclusion that the maximum fusion pore aperture corresponds to ca 10 degrees, i.e., evidencing that some biological local structure opposes full fusion.
Research on interfacial nanobubbles has greatly advanced during the past decade, including the development of new methods of generating and detecting nanobubbles, as well as the development of the theory and mechanism of nanobubble formation and stabilization. At present, it is possible to generate large ensembles of nanobubbles of different gas types at hydrophobic surfaces (e.g. perfluorodecyltrichlorosilane and highly orientated hydrophobic pyrolytic graphite using the solvent exchange technique or by the electrolysis of water.

In this report, we present a new approach for investigating the formation and stability of a nanobubble. Instead of generating a large ensemble of nanobubbles at a macroscopic surface, a Pt nanodisk electrode (< 50 nm radius) is used to electrochemically generate a single H2 or O2 nanobubble by water reduction or oxidation in a strong sulfuric acid solution. The nanoscale dimension of the nanoelectrode itself provides exquisite sensitivity for detecting small changes near or at the electrode surface, while fast electrochemical measurements allow study of the dynamics of nanobubble formation. High spatial and time resolutions make the nanodisk electrode a powerful platform to study the formation and stabilization of nanobubbles. We demonstrate that 10 nm radius bubbles, with internal pressures of 150 atm, are stable at the surface of a Pt nanodisk electrode.

Abstract Text

Keywords: Electrochemistry, Electrode Surfaces, Electrodes
Application Code: Nanotechnology
Methodology Code: Electrochemistry
Electroanalytical Chemistry on the Nanoscale

Nanostructured Microfluidic Arrays for Protein Detection and Genotoxicity Screening

Nanotechnology and microfluidics provide exciting opportunities for multiplexed bioanalytical devices. Nanostructured platforms in electrochemical biosensors enhance performance over flat electrodes, partly because they accommodate high surface concentrations of sensor materials. We developed modular microfluidic systems for ultrasensitive detection of multiple proteins and for toxicity screening. For protein detection, a 100 [micro]L chamber for on-line protein capture is positioned upstream of a nanostructured multi-sensor array in a 60 [micro]L chamber. Amperometric multi-sensor array chips were fabricated by ink-jet printing of 4 nm gold nanoparticles ($0.20/chip), commercial screen printing of carbon and coating with 5 nm gold nanoparticles ($9/chip), wet-etching of gold CDs, or, for ECL, microwell-patterning of pyrolytic graphite ($0.25/chip). A print/heat/peel method transfers computer printed patterns onto arrays to create hydrophobic wells around each sensor to facilitate building active sensing surfaces without cross-contamination. Arrays are fitted into a PDMS microfluidic detection channel, and attached to a syringe pump. Approaches feature massively labeled particles to amplify signals. Detection limits as low as 5 fg/mL were achieved for simultaneous measurement of four oral cancer biomarker proteins in a few [micro]L of serum. An electrochemiluminescent (ECL) chip was also designed to screen genotoxicity-related chemistry of drug and pollutant metabolites. We printed 64 1.0-[micro]L microwells onto a pyrolytic graphite chip, and incorporated it into a microfluidic device. Films combining DNA, metabolic enzymes and a ruthenium ECL polymer were built on carbon nanotube forests grown in the microwells. The system runs metabolic reactions, then detects DNA damage caused by reactive metabolites via increased ECL measured by a CCD camera. The device was tested by measuring DNA damage caused by benzo[a]pyrene.

Keywords: Array Detectors, Biological Samples, Biosensors
Application Code: Bioanalytical
Methodology Code: Electrochemistry
Electroanalytical Chemistry on the Nanoscale

Electrochemical Nanoprobes for Analysis and Mechanistic Studies

Nanometer-sized electrodes and nanopipettes have recently been used to investigate important chemical and biological systems. Although electrochemical nanosensors offer a number of advantages, visualization of their surfaces remains challenging. Thus, the interpretation of the electrochemical response relies on assumptions about the shape and size of the nanoprobe prior to the experiment and the changes induced by surface reactions. In this paper, we discuss the use of nanometer-sized electrodes and pipettes in combination with scanning electrochemical microscopy (SECM) and AFM for mechanistic studies of surface reactions and electroanalysis on the nanoscale.

Keywords: Electrochemistry, Microelectrode, Nanotechnology, Small Samples

Application Code: Nanotechnology

Methodology Code: Electrochemistry
Development of New Extraction and Analysis Methods for the Rapid Detection of Characteristic Chemicals from Humans and Contraband Materials

This presentation describes recent developments in field sampling methods and hyphenated analytical techniques developed for the rapid detection and identification of characteristic chemicals from persons and illicit substances. Methods employed include dynamic headspace concentration, SPME and fabric phase sorptive extraction (FPSE) followed by GC or LC separation and mass spectrometric detection to identify characteristic organic compounds. Fast and efficient analytical methods have been developed for the simultaneous extraction and analysis of a wide range of analytes using FPSE medium bonded with sol-gel sorbent. Various FPSE parameters have been optimized and it is demonstrated that FPSE provides for the detection of ultra-trace levels of analytes from different sample matrices. Using optimized dynamic headspace sampling the chemical profiles of living human samples are compared and contrasted to deceased human samples and human cadaver analogues under various environmental conditions. The dynamic headspace sampling method proved to be a useful field instrument for identifying human derived VOCs and also for the preparation of canine training aids. Identified odorants have been used to develop odor mimics for use as field calibrants for biological and electronic detectors with a limited number of mimics able to represent a wide range of illicit substances. These mimics are useful in comparing thresholds of detection and improving the sensitivity and reliability of detection. Detection limits are compared for different biological and instrumental detectors. The use of a universal calibrant has also been shown to be useful for initial testing and threshold testing across all types of biological detectors, including dogs and birds, as well as electronic sensors.

Keywords: Extraction, Forensics, Forensic Chemistry, SPME
Application Code: Bioanalytical
Methodology Code: Sampling and Sample Preparation
In forensic case work chemical analysis of explosives, associated precursors and post-explosion residues usually aims at compound identification to determine the type of explosive that was made or used. While forensic chemical impurity profiling is a methodology that is used frequently in the forensic investigation of illicit drugs to study manufacturing, transport and trade, it is not used often in forensic explosive analysis. Methods to determine whether materials are related are not used as much in forensic explosives analysis mainly because of three reasons:

1) The number of crimes involving explosives on a national and global scale is very small compared to the size of drugs-related crimes
2) There has been less focus by the law enforcement community on having such profiling methods available for explosives because of limited international trade compared to illicit drugs.
3) Explosives and precursors span a wide variety of materials and mixtures both organic and inorganic, stable and labile. Consequently, for explosives in contrast to illicit drugs no universal analytical methodology exists.

Despite these complications chemical profiling of explosives can be very valuable even in a stand-alone case. If the same type of explosive that was detected at the crime scene is also found in the home of a suspect this fact in itself does not provide strong evidence that links the suspect to the crime.

Based on these insights the Netherlands Forensic Institute started in 2009 with a PhD study on forensic chemical profiling of explosives in close collaboration with the University of Amsterdam and TNO. In this presentation an overview of the results of this work will be given and three novel methods will be introduced:

1) TNT impurity profiling with vacuum outlet GC-MS
2) PETN profiling in post explosion residues with LC-HR-MS
3) Ammonium nitrate profiling with IRMS and ICP-MS

Keywords: Forensics, ICP-MS, Isotope Ratio MS, Liquid Chromatography/Mass Spectroscopy
Application Code: Homeland Security/Forensics
Methodology Code: Other (Specify)
The objective of this talk is to present results of research designed to determine if decontamination can be performed to provide the accepted level of log reduction without destroying the ability of DNA to be analyzed by standard assays. The research was divided into 3 parts. In the first part, a variety of decontaminating agents are used to kill spores of B. cereus, a surrogate of the pathogen B. anthracis, deposited on common office space materials. After spore recovery, the DNA was extracted and tested for activity in the polymerase chain reaction (PCR) and with pulsed field gel electrophoresis. In the second part, studies were performed to determine if DNA of vegetative cells (E. coli, S. aureus, A. faecalis, S. typhimurium, and S. pyogenes) that act as surrogates for various pathogens, deposited on a non-porous surface and exposed to chlorine dioxide gas, can be active in the PCR, restriction digestion and sequencing reactions. In the third part, studies to demonstrate if the DNA (RNA) from live agents (B. anthracis Ames, B. anthracis Vollum, B. anthracis Sterne, Coliphage MS2, Venezuelan Equine Encephalitis virus), deposited on non-porous surfaces either as a liquid slurry or as a powder and exposed to chlorine dioxide gas, is active in molecular assays. Overall, we found that it is possible to simultaneously decontaminate and exploit contaminated sites for microbial DNA evidence. Decontaminating agents can be selected that do not interfere with the nucleic acid (DNA/RNA) detection assays. Post-decontamination preservations of DNA (RNA) allow the demonstration that culture-negative samples have been collected.

Keywords: Environmental/Biological Samples, Forensics, Nucleic Acids
Application Code: Homeland Security/Forensics
Methodology Code: Other (Specify)
High Efficiency Sampling Using Capillary Microextraction of Volatiles (CMV) Coupled to Gas Chromatography – Mass Spectrometry (GC-MS)

A novel geometry configuration based on sorbent-coated glass microfibers packed within a glass capillary is used to sample volatiles, dynamically, in the headspace of an open system or in a closed system to achieve quantitative extraction of the available volatiles with negligible breakthrough. Air is sampled through the newly developed sorbent-packed 2 cm long, 2 mm diameter capillary microextraction of volatiles (CMV) and subsequently introduced into a commercially available thermal desorption probe fitted into a GC injection port. A sorbent coating surface area of \( \sim 5 \times 10^{-2} \) m\(^2\) or 5000 times greater than the sorbent coating of a single SPME fiber, allows for fast (30 s), flow-through sampling of relatively large volumes using sampling flow rates of \( \sim 1.5 \) L/min. A direct comparison of the new CMV extraction to a static (equilibrium) SPME extraction of the same headspace sample yields a 30 times improvement in sensitivity for the CMV when sampling nitroglycerine (NG), 2,4-dinitrotoluene (2,4-DNT) and diphenylamine (DPA) in a mixture containing 500 ng total mass of each analyte, when spiked into a liter-volume container. Calibration curves were established for all compounds studied and the recovery was determined to be \( \sim 1\% \) or better after only 1 min of sampling time. Quantitative analysis is also possible using this extraction technique when the sampling temperature, flow rate and time are kept constant between calibration curves and the sample.

Keywords: Sample Introduction, Sampling, SPME
Application Code: Homeland Security/Forensics
Methodology Code: Sampling and Sample Preparation
Identifying biological fluid stains on materials of evidentiary value (e.g., clothing from a crime scene) is a critical task. Many sensitive methods risk contaminating DNA evidence; the most nondestructive are also the least sensitive. We have developed infrared imaging approaches to visualize blood and bodily fluids on fabrics and other substrates. The ideal device would be small, inexpensive, easy to operate, portable, useful indoors or outdoors under ambient lighting, and nondestructive; enhancement reagents would not be necessary; further, detecting trace levels of blood is desired. Fabrics adsorb/desorb water depending on humidity, and we observed differential contrast in the thermal response of bloodstained and clean fabrics on exposure to water vapor generated from a hand-held steamer. We call this method latent heat thermography (LHT) imaging because it is related to the enthalpy of adsorption of water vapor. The mechanism of visualization is the differential rate and extent of vapor absorption. The present scope of our has been to find conditions that help best visualize bloodstains on fabrics, and to understand the mechanism(s) by which dilute bloodstained fabric is distinguished from unstained fabric.

We tested a dyed unpatterned acrylic fabric and a patterned black polyester. Undiluted blood, and 10- or 25-fold diluted blood can be seen. However, 50-, 100- and 1000-fold dilutions of blood are not visible. Alternate light sources for blood detection detected blood stains at 100 X dilution on the acrylic, but the stains on patterned polyester were not detected, even knowing that they were present. All stains can be easily visualized by steaming with water vapor, illuminating the target with IR light, and observing the light reflected from the samples. Different adsorption-desorption rates are observed between blood and other substances which commonly give false positives. This novel method could have broad applications in imaging of trace evidence.

**Keywords:** Forensics, Infrared and Raman, Vibrational Spectroscopy

**Application Code:** Homeland Security/Forensics

**Methodology Code:** Vibrational Spectroscopy
The metabolome, or the total compliment of small molecules in a living system that includes endogenous and introduced compounds, reflects the overall global biochemical state of an organism. These small molecules are produced by and interact with proteins and thus changes in the functional genome are closely tied to changes in the metabolome. Metabolomics (or metabonomics) is the comprehensive measurement the metabolome and how it changes in response to external stressors. In Pharmaceutical R&D, this information can be used deduce the relationship between a perturbation (such as disease or pharmacological intervention to disease) and the effected biochemical pathways. These are closely linked with molecular changes that correspond to the nature of the perturbation. If the association is biochemically relevant and the molecular measurements robust, the molecule or molecules that change can be used as biomarkers of the event and can be used to inform and accelerate the discovery of safe and efficacious drugs. The field of metabolomics has grown considerably over the past 15 years and mass spectrometry and nuclear magnetic resonance spectroscopy have become front-line tools in evaluating the metabolome. This talk will provide a background on the technology and present several examples of how it has been employed to discover biomarkers in mainstream pharmaceutical R&D.
Mass spectrometry (MS)–based proteomic approaches have become increasingly popular tools for biomarker discovery. However, the identification of potential protein biomarkers from biofluid samples remains to be challenging because of the extreme sample complexity and limited dynamic range of detection. This presentation will focus on our recent progress in the development and application of MS tools for biomarker discovery in two neurodegenerative diseases, namely Alexander disease (AxD) and Alzheimer’s disease (AD), using cerebrospinal fluid (CSF) samples. CSF samples obtained from a mouse model for AxD were analyzed via a shotgun proteomics approach. This approach resulted in the identification of 289 proteins with relative quantitation of 103 proteins performed using label-free spectral counting analysis. In a more recent study of biomarker discovery in AD patients, we conducted a large-scale comparative glycoproteomic analysis via lectin affinity chromatography to enrich glycoproteins from CSF samples collected from control, mild cognitive impairment (MCI) and AD groups. Our preliminary results showed that 137, 145 and 132 glycoproteins were identified in control, MCI and AD group respectively by spectral counting. Among these proteins, 75 identifications show increasing or decreasing trend from control – MCI – AD, which are potential biomarker candidates that may play a significant role in diagnosis and treatment of AD. Furthermore, endogenous peptidome of these CSF samples were also examined for the first time to reveal potential disease biomarkers. Finally, novel dimethylated leucine (DiLeu) isobaric tagging reagents were developed and evaluated for their utility in multiplexed quantitative analysis of these putative biomarkers.

**Keywords:** Biomedical, Mass Spectrometry, Proteomics, Quantitative

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

**Methodology Code:** Mass Spectrometry
Pharmaceutical industries have invested heavily in the search for novel drugs, including small molecules and biologics for the treatment of life-threatening diseases. However, the drug attrition rate in clinical trials continues to rise. Recent advances in translational medicine have led to a better understanding of biological pathways and enabled a better means of establishing the efficacy and safety of therapeutic interventions in both preclinical and clinical studies. The presence of species specific sequences and protein variants in biological samples has increased the interest in augmenting ligand binding assays with the molecular specificity of mass spectrometry. This presentation will discuss the integrated strategy in combining immunochemistry with LC/MS methodologies for the quantitative analysis of protein biomarkers including case studies.

**Keywords:** Bioanalytical, Biological Samples, Drug Discovery, Liquid Chromatography/Mass Spectroscopy

**Application Code:** Bioanalytical

**Methodology Code:** Liquid Chromatography/Mass Spectrometry
Immunoassays are currently the gold standard for clinical protein measurement, although they have limitations, primarily with respect to antibody specificity. Quantitative measurements using traditional immunoassay methods, such as ELISA, are often affected by components in the sample matrix and other confounding factors. Furthermore, they are often unable to distinguish among sequence variants and PTM heterogeneity of the target proteins. Mass spectrometry (MS) has been used for quantification of small molecules in clinical laboratories for years, and, more recently, has been applied to protein quantification and analysis. Because MS can resolve proteins at the sequence level, it provides the selectivity to address the issues outlined above.

The large dynamic range of proteins in serum/plasma samples compromises the ability of MS to achieve sufficient sensitivity (signal-to-noise ratio) for accurate quantification of many clinically important markers and therefore requires prior enrichment of the less abundant species. In addition, many disease-related proteins are either truncated, modified by post translational modifications (PTM’S) or single nucleotide polymorphisms (SNP’s) and are present in multiple active and inactive isoforms. It is imperative in many cases to measure not just total protein concentration but also relative concentrations of clinically relevant isoforms in order to gain the necessary specificity in protein assays. Recently, we reported the development of robust, high-throughput, quantitative MS assays for parathyroid hormone (PTH), PSA, insulin and other clinically important analytes. The described method coupled a previously developed technique for immunoenrichment on a monolithic microcolumn activated with an anti-protein antibody and fixed in a pipette tip (MSIA), to detection using high resolution LC-MS/MS. In this presentation, we describe the MSIA-MS workflow and present data for a variety of different, clinically important analytes.

Keywords: Mass Spectrometry, Peptides, Plasma, Protein
Application Code: Biomedical
Methodology Code: Mass Spectrometry
Abstract Text

Food safety is everyone’s responsibility within industry from farm to fork. However, until recently food safety risk management programs were encouraged but not enforced. Through the Food Safety Modernization Act (FSMA) the FDA has now issued ruling to regulate produce. These regulations emphasize employee training, health and hygiene, agricultural water, biological soil amendments of animal origin, domesticated and wild animals, equipment, tools and buildings. While this initiative strives to reduce food safety risks, it will introduce some challenges in implementation on-farm.

As part of the proposed rules, farmers will soon be responsible for validating the food safety of their on-farm water and soil. Part of the proposed ruling is to have agricultural water tested routinely to ensure that the water source is safe for its intended on-farm use. If the tested water fails the declared compliance, certain actions must be taken to make it safe (proposed sections 112.44 and 112.45). While these activities are intended to support the reduction of foodborne pathogens within produce, they will have a significant impact on many farmers’ methods of growing produce.

While demand has been growing for the consumption of fresh produce for better health and nutrition, at present, a practical non-thermal process to reduce pathogenic risk in produce has not been put into practice. Food safety has continued to grow in importance, and the climate is changing to demand that stronger food safety programs are instituted throughout the food chain from farm to fork.

This talk will review the ruling pertaining to produce food safety, the impact that this will have on farms, share what the upcoming extension support efforts are to ensure successful transition to stakeholders when final rulings are passed and discuss the need for rapid low-cost diagnostic assays for on-farm use.

Keywords: Agricultural, Biological Samples, Water
Application Code: Agriculture
Methodology Code: Other (Specify)
Dairy farming is an important source of income for small holder farmers (SHFs) in sub Saharan Africa. The small herd size (2-4 cows) of SHFs, however, makes it difficult to detect estrus using traditional behavioral monitoring methods. This can lead to cows spending time in an ‘open’ (i.e., non-pregnant) state, which results in lost income for the farmer. Diagnostics For All is developing a low-cost, paper-microfluidic test that detects estrus in cows from an ear or tail-stick drop of blood. The test will enable SHFs to more reliably determine when a cow is ready for artificial insemination, which will increase conception rates, milk production, and income, and will reduce costs associated with poorly timed artificial insemination attempts. This talk will describe recent field demonstration results where bovine serum samples were tested on device prototypes in Kenya, and it will also cover progress towards the development a blood-based point-of-care test for on-farm diagnosis.
On-Farm Diagnostics for Improved Food Safety, Quality, and Production

An On-Farm Device for the Detection of Generic E. coli from Agricultural Water Sources

The FDA has recently published rules to improve on-farm microbial safety for produce typically consumed raw by the consumer, with a focus on agriculture water quality. The rules require farmers to regularly monitor generic E. coli as an indicator of water quality, with a sample limit 235 cfu/100 ml and a rolling 5-sample mean of 126 CFU/100 mL. To meet these requirements farmers will need methods that are reliable, rapid, cost-effective, and easy-to-use. Lateral Flow Assay (LFA) test strips have successfully been used to address these challenges in areas ranging from allergen to pregnancy testing. Our work focuses on combining phage-based signal amplification with an LFA strip to detect generic E. coli. T7 phage is utilized due to its broad specificity for E. coli, short infection cycle, and relatively large burst size allowing for a 100-fold increase in the signal of a single E. coli cell. The LFA strip is a sandwich based assay utilizing antibodies for the T7 phage and a fluorescent nanoparticle reporter. A sample containing E. coli cells is inoculated 10^3 PFU/mL of T7 phage for 90 minutes, and then a 30 μl aliquot is tested on the LFA. Our data shows that after incubation, the final level of T7 phage in the sample is proportional to the initial number of E. coli cells present in the sample (R^2 = .96), and that a level of 10^3 CFU/100 mL of E. coli must be present to produce sufficient phage signal above our initial inoculation. Our data also shows that the LFA can detect a level of 10^4 phage PFU/mL of sample in 30 minutes. These findings suggest that a phage-based LFA device offers a solution for on-farm testing of agricultural water. Optimization of sample concentration, such as filtration or IMS, should allow the method to meet required bacterial sensitivity.

Keywords: Agricultural, Detection, Environmental/Water, Food Science

Application Code: Agriculture

Methodology Code: Microfluidics/Lab-on-a-Chip
Current field portable detection technologies can be cumbersome and require generous quantities of chemicals to operate. Designing resistance biosensors based on the use of nonwoven fiber membranes coated with conductive polymer coatings and functionalized with antibodies provides the source for the development of small handheld devices for the biological capture and detection of pathogens in the field. These functionalized electrotextiles can be produced by conformally coating the nonwoven membranes with either polypyrrole or PEDOT either by an aqueous deposition process or by oxidative chemical vapor deposition (oCVD). Inclusion of a copolymer containing either a carboxylic acid or hydroxyl functional group, such as 3-thiophene acetic acid or 3-thiopheneethanol, provides functionality for covalent attachment chemistries of pathogen specific antibodies. The functionalized electrotextile membranes can perform as both the capture platform and conductive medium for resistance based detection. After exposure, the fiber discs are extracted from the sample, washed with phosphate-buffered saline (PBS), and placed between two electrodes. The electrodes are then sandwiched together with the functionalized fiber disc acting as the electrical conductor between them. A current is run through the system, the presence of the captured pathogen on the fiber surface results in an increase in resistance between the two electrodes, indicating a positive result.
Thinking Outside the Laboratory: Innovative Outreach and Educational Approaches that Bring Analytic

Bringing Instrumental Analysis into the K-12 Classroom: Service Learning Projects and Laboratory Coursework

Service learning engages students with their community by connecting curricular material with community service. Laboratory coursework, which is already an active, hands-on learning environment, presents an excellent opportunity for students to engage with their community. In Fall 2011, my instrumental analysis laboratory class performed SPE-GC-MS and ELISA determinations of pesticides in water from a local river. In lieu of a lab report, the students shared the results and significance of their analyses with fifth grade classes at Rankin Elementary School. The undergraduate students wrote an abstract and a lesson plan based on their experiments. They also hosted over 100 fifth graders on a field trip to the Chemistry Department, where the fifth grade students conducted a simulated ELISA experiment. In Spring 2014, my instrumental analysis class at Trinity College is partnered with sixth graders at Hartford Magnet Trinity College Academy (HMTCA) in a similar project. The HMTCA students will collect samples and perform water quality testing using chemical tests strips, then share their samples with Trinity undergraduates for determination of trace metals by ICP-OES. These service learning projects give students an opportunity to practice presentation skills and master material by teaching it, and they introduced a diverse group of schoolchildren to successful college science students. Based on my experience, I will present practical aspects of incorporating service learning into a laboratory class, including identifying overlap in learning objectives for instrumental analysis students and state K-12 science standards, designing assignments and assessments, and obtaining funding and resources.

Keywords: Education, Environmental Analysis, Environmental/Water, Teaching/Education
Application Code: General Interest
Methodology Code: Education/Teaching
Analytical technology can enhance middle school science education by enabling students to take charge of scientific research. To implement this practically, the technology must be accessible to the student as well as the educator. Microfluidic devices provide an innovative alternative to traditional science education. Electrolysis of water is a classic chemistry experiment normally conducted with bench top equipment designed for group presentation. Safety considerations relevant to middle school students include the generation of hydrogen and oxygen gas. By reducing the volume from 100 milliliters to 100 microliters the electrolysis cell is easily and safely viewed by an individual. This is significant to middle school education because hand held equipment personalizes the process of scientific discovery through kinesthetic learning. A single device is reusable and can be built for under 20 USD. Curriculum to accompany these devices has been developed to meet content standards and objectives in West Virginia. In this presentation we describe the development, implementation, and assessment of the microfluidic learning module. Finally, the dissemination of the materials through workshops is described.

Keywords: Education, Lab-on-a-Chip/Microfluidics
Application Code: Other (Specify)
Methodology Code: Microfluidics/Lab-on-a-Chip
Laboratory projects that require students to solve real problems for local businesses were implemented in an analytical chemistry course at Indiana University. The change from a traditional curriculum to active learning projects was driven by shortfalls in course objectives. Specifically, the projects were designed to increase student engagement, improve accuracy and precision of measurements, strengthen scientific communication skills, and develop expertise with instrumentation. Guidelines for leading students through a six phase process for problem solving and instructional adaptations for an active learning environment will be presented. Details of a quality assurance project developed in collaboration with a local microbrewery, strategies for implementing projects in large classes, and student outcomes will be provided.
Abstract Text

The unambiguous and ultrasensitive identification of dyes and pigments is exceedingly important for the conservation of works of art. Although current technology provides conservators with the ability to identify inorganic colorants, the detection of organic materials, those most prone to fading and decomposition, has remained challenging. In an effort to improve current analytical methodologies for colorant identification and to enhance the research experience of undergraduate students at The College of William and Mary, a new collaboration with the paintings conservation department at the Colonial Williamsburg Foundation has been established. In this collaboration, we have developed novel strategies for the identification of fugitive organic pigments in historic oil paintings. Correlated SERS and fluorescence microscopy measurements on single pigment grains are used to identify carmine lake in two 18th century oil paintings, the [i]Portrait of Isaac Barre[/i] and [i]Portrait of William Nelson[/i]. Pretreating art samples with sulfuric acid enabled the SERS-based identification of insoluble indigo, Prussian blue, and mixtures thereof, in aged painted surfaces. For example, a microscopic acid-treated sample from the Portrait of Evelyn Byrd produced a SERS spectrum that is consistent with a mixture of Prussian blue and indigo. In addition to advancing scientific objectives, this collaboration provides a unique opportunity for students to experience “behind the scenes” of the conservation world. This talk will discuss the process of partnering with local conservation groups, our educational and research outcomes, as well as SERS-based analysis of paintings.

Keywords: Art/Archaeology, Molecular Spectroscopy, Nanotechnology, Raman
Application Code: Art/Archaeology
Methodology Code: Vibrational Spectroscopy
Advances in Sensor Technology for Food Safety and Food Quality

Measurement of Trichothecene Mycotoxins in Wheat Using a Biolayer Interferometry-Based Biosensor

Mycotoxins are secondary metabolites produced by fungi. The fungi can infest a variety of important agricultural commodities including wheat, barley, maize, peanuts, and tree nuts. Certain of the mycotoxins are potential threats to animal and human health and, for this reason, extensive monitoring is done of their presence. Among the mycotoxins, trichothecenes are a heterogeneous group of sesquiterpenoids that are acutely toxic to many species. As part of efforts to improve detection of trichothecenes, a biosensor based upon biolayer interferometry (BLI) was developed to detect two of the trichothecenes: deoxynivalenol and T-2 toxin. The sensor has the advantage that it allows for the monitoring of binding events in real time. Also, rather than forcing test solutions through fine capillaries, which can clog, this device uses sensors that are dipped into the test solution, eliminating this potential problem. The technique was successfully used to measure T-2 toxin and deoxynivalenol in spiked whole wheat flour at levels below current regulatory guidelines. These results suggest the technique may be useful for the rapid identification and quantification of trichothecenes in commodities and foods.

Abstract Text

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Keywords: Agricultural, Biosensors, Immunoassay, Toxicology
Application Code: Agriculture
Methodology Code: Sensors
The USDA has recognized that food-borne illness can be caused by a number of Shiga-toxin-producing Escherichia coli (STEC) strains besides the well-known O157:H7 serotype. Based on the risks associated with STEC, the FDA has banned the presence of seven STEC serotypes from beef: O26, O45, O103, O111, O121, O145 and O157:H7. The expansion of testing requirements to include non-O157 STECs has created a greater need for sensitive, multiplexed assays that can detect multiple pathogenic organisms in food matrices. In this talk we will discuss the application of the Meso Scale Diagnostics (MSD®) electrochemiluminescence (ECL)-based detection technology to the multiplexed measurement of food pathogens. In one example, we will demonstrate the use of the MSD detection platform and a set of serotype-specific polyclonal antibodies from "SeraCare Life Sciences (formerly Kirkegaard & Perry Labs) for the simultaneous detection of all seven banned STEC serotypes. The pathogens are detected in a sandwich immunoassay format using arrays of antibodies printed in each well of a 96-well plate. The protocol uses a single incubation step and has a rapid – 1 hour – time-to-result. The array-based approach will allow for future expansion of the panel to include other STECs or non-STEC pathogens.

Keywords: Array Detectors, Food Science, Immunoassay, Industrial Hygiene
Application Code: Safety
Methodology Code: Bioanalytical
Advances in Sensor Technology for Food Safety and Food Quality

Application of IR Chemical Imaging and DNA Microarrays to the Identification of Fish Species

The FDA is responsible for the safety of the US seafood supply, a mandate that requires the development of high-throughput methods for regulatory screening. While morphological indicators may help identify whole, unprocessed finfish species, the visual identification of processed seafood has been challenging. DNA-based methods are needed to correctly identify processed fish species. This is because the large diversity of imported seafood species marketed in the US makes it difficult to prevent species substitution, economic adulteration, and misbranding. Emerging methods such DNA microarrays have recently been proposed for their potential to confirm the identity of closely related fish species.

In the present proof of concept, an infrared imaging assay for the discrimination of commercial catfish species using a spotted glass microarray format with silver augmentation has been developed. According to US rules, only Ictalurus species may be called “catfish.” Two of the Pangasius species, P. boucourti and P. hypothalamus, that should be imported as Basa and Swai respectively, are often misbranded as “catfish” for economic gain. Using DNA microarrays it was possible to discriminate between Ictalurus and Pangasius species. Infrared imaging and Raman depth profiling data will be presented and discussed.

Keywords: Food Science, FTIR, Imaging, Raman
Application Code: Food Science
Methodology Code: Sensors
Foodborne diseases caused by Escherichia, Listeria, and Salmonella species affect as many as 50 million people in the United States each year, resulting in 130,000 hospitalizations and over 3,000 deaths (1996-2010). 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. Here we present SERS measurements of these pathogens in actual food samples using this assay.
Advances in Sensor Technology for Food Safety and Food Quality

Identification of Microorganisms by Raman Spectroscopy for the Development of New Biosensors in the Food Industry

Microbial food safety is a major concern for all contributors in the food chain. The regulations require systematic controls to ensure the innocuousness of products by analytical methods. How can we reconcile the need to perform more and more tests in a relatively short time everywhere in the world with the need for cheaper and accurate methods?

One possible answer is the use of Raman spectroscopy. This vibrational method, provides in a very short time a spectrum of the bacteria which is a fingerprint of its chemical composition. Even though the possibility of using the technique for the characterization of bacteria is well established, the problem has yet to be solved within the constraints of the food industry. Following the current Salmonella sp standard protocol, we applied Raman technology having in mind to reduce the time for analysis but also to propose a reproducible method.

Indeed, the quality of the spectrum depends on the physiological state of the bacteria. We analyzed by Raman spectroscopy several strains according. The classification of more than 1000 spectra by statistical methods shows that the exponential growth phase is the best stage for the discrimination. A rigorous protocol was designed and validated with many samples from food industry.

A second main concern when applying Raman spectroscopy is the reliability of the methods considering the process of pre-enrichment and growth of the bacteria. We considered the choice of several Pepton Broth Water (PBW) media from different manufacturers and we addressed the problem of the variability of their compositions on the Raman spectrum. It was demonstrated that all the BPW are not suitable for pre-enrichment leading to different microbial kinetics as well as differences in their Raman spectra.

The final goal will be to introduce this new biosensor into the control system of the food production chain thanks to a reliable database for various microorganisms.

Keywords: Bioanalytical, Biosensors, Identification, Raman
Application Code: Food Science
Methodology Code: Vibrational Spectroscopy
Piezoelectric cantilever sensors are shown to exhibit sensitive and selective detection based on both an antibody binding reaction as well as by hybridization of an identifying gene from genomic extract at 102–103 cells of foodborne pathogen, Listeria monocytogenes (LM). The study consists of two parts: tests with synthetic genes and experiments starting with whole LM cells. A probe designed for the virulence hemolysin gene, hlyA, was immobilized on the gold-coated sensor, and hybridization detection of a synthetic target (based on hlyA) is shown to span over 6 decades in concentration. Hybridization response was confirmed using two methods: (1) the use of a fluorescent indicator for the presence of double-stranded DNA (ds-DNA) and (2) hybridization response of a secondary single-strand DNA (ss-DNA) to the unhybridized part of the target much like in the enzyme linked immunosorbent assay (ELISA) sandwich format. Hybridization of the secondary ss-DNA tagged to gold nanoparticles amplified as well as confirmed the target hybridization to the hlyA probe on the sensor. Genomic DNA from LM was extracted, sheared, and melted and was exposed to the hlyA probe on the sensor in proteinous background with and without the presence of up to 104 times excess nontarget genomic DNA extracted from E.coli JM 101 (EC), for the gene-specific detection of LM. Discernible detection limit of 7 × 102 LM cells (equivalent genomic DNA; 2.32 pg) was achieved in proteinous background. The detection limit deteriorated to 7 × 103 LM (23 pg of gDNA) in the presence of genomic DNA from EC. Hybridization response times were within 90 min, thus significantly improving over the conventional detection techniques in detection time at comparable detection limit.
Market demands for new sensors for food quality and safety stimulate the development of new sensing technologies that can provide an unobtrusive sensor form, battery-free operation, and minimal sensor cost. Intelligent labeling of food products to indicate and report their freshness and other conditions is one important possible application of such new sensors. This study applied passive (battery-free) radio frequency identification (RFID) sensors for the highly sensitive and selective detection of food freshness and bacterial growth.
Abstract Text

Non invasive optical methods for food analysis are the next generation of assays bringing a large number of data in a very small time. The main troubleshooting is the accumulation of a huge data number that chemometrics is able to interpret. Chemometrics is the discipline that combines data analysis and analytical chemistry. It brings together and develops a set of mathematical and statistical tools used to extract structured and interpretable information from chemical data. The use of chemometric tools has spread incredibly over the two last decades because users understand how these tools could save them time and money. Moreover, in some cases, certain types of data such as infrared or nuclear magnetic resonance spectroscopy, chemometric tools are almost mandatory to achieve a useful and effective treatment of the chemical information.

We quickly introduce Independent Component Analysis - ICA and interval-Partial Least Squares – iPLS which are tools to reduce the dimensionality of the data while improving the interpretability of data and build robust and efficient calibration models. In the case of 3 Dimensional-Front Face fluorescence spectroscopy (3D-FFFS) which will be discussed in an example, the nature of the data impose the use of the specific mathematical tools for data manipulation and model calculation. The presented example deals with the detection of adulteration of honey by industrial sugar syrup by using 3D-FFFS. When the chemometrics is coupled with microRaman spectroscopy and microfluidic analytical systems, the result is a new and rapid tool for the concentration determination of chemical compounds from organic mixtures and bacteria detecting and counting in food. Two others examples show the automation of these analyses and how the chemometrics help the user to masterize his analysis and adjust his decision in order to better manage the quality of food.

Keywords: Chemometrics, Fluorescence, Lab-on-a-Chip/Microfluidics, Raman
Application Code: Food Science
Methodology Code: Chemometrics
Simulation of the chromatographic process by repeated equilibration and movement steps is readily possible in Microsoft ExcelTM (1). More recent versions of Excel also permit the color gradation with 4-bit resolution of numerical values in any series of numbers (highest value connoting densest coloration) permits visualization of a band as it moves down a column, much as Tswett observed chromatographic separation of the chlorophyll components for the first time. The more often one plays with how chromatographic bands are moving through a column, the more one realizes how often the separation is complete long before it reaches the end-column detector. In cases where steep eluent gradients are applied, resolution of two poorly resolved analytes may have been better at the column compared to when they appear at the detector. We become very wistful to have X-ray vision to look at the separation as it develops like Tswett experienced, except to have a quantitative vision. Attempts have been made towards whole column imaging in optical detection, especially in fluorescence based isotachophoretic systems. We present dynamic conductivity imaging of an open tubular column that quantitatively images the temporal development of ionic separations. Time permitting, optical detection will also be discussed.


Keywords: HPLC Detection, Ion Chromatography
Application Code: General Interest
Methodology Code: Liquid Chromatography
The utility of a suppressor in ion chromatography is well established. The original suppressors were based on a packed bed of ion exchange material which required regeneration. The development of a continuously regenerated suppressor paved the way to a robust analytical platform for pursuing ion analysis. Advances in regeneration such as by the use of electrolysis resulted in a more easy to use suppressor system. Further the recycle mode of operation made it even more convenient and for the first time ion analysis was feasible with just an eluent containing reservoir coupled to an ion chromatograph. Advances in the eluent generation technology resulted in a system that today could be operated with deionized water.

In the first part of this presentation we review the suppressor technology from a historic perspective. We will discuss the evolution of the suppressor from a packed bed to a continuously regenerated suppressor. In the second part of this presentation we discuss a newly introduced electrolytic suppressor called the Electrolytically Regenerated Suppressor (ERS). We will discuss the design details along with the key benefits. We will also show example performance characteristics of the new suppressor. Results from analyzing real life samples such as drinking and waste waters will also be shown here.

Keywords: Instrumentation, Ion Chromatography, Ion Exchange, Water
Application Code: High-Throughput Chemical Analysis
Methodology Code: Other (Specify)
The quality of the drinking water is essential to the public health and is currently subjected to various federal and state regulations. Ion Chromatography with suppressed conductivity detection has been widely utilized to monitor the ionic species in drinking water. However, many of the ionic contaminants of interest can be present at a very low concentration level such as in the low µg/L range, while some of the common ions can be present at high concentrations typically in the mg/L range. This poses an analytical challenge particularly from a separation resolution perspective and in some occasions the resolution between the analytes of interest and the common ions may be inadequate and therefore posing a quantitation challenge for the peaks of interest.

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 to isolate the analytes from the common ions in the first dimension. Since the suppressed effluent is essential deionized water, the portion containing the analytes of interest can be re-analyzed 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, Environmental Analysis, Ion Chromatography
Application Code: Environmental
Methodology Code: Chemical Methods
Ion chromatography (IC) is one of the most reliable techniques for analyzing a wide variety of ions and molecules of biological interest. Four decades since its invention, IC stationary phases are still dominated by long columns (20-25 cm) and large particle diameters (7-13 [micro]m). In 2011, we saw the emergence of capillary columns packed with 4 [micro]m particles, but the columns are still long (15-25 cm). In contrast, reversed phase separations routinely use sub-2 [micro]m silica particles in short columns in ultrahigh performance liquid chromatography (UHPLC). The separation often takes less than a minute. Unfortunately, silica cannot handle the alkaline eluents used in IC. Still, practitioners have naturally begun to ask “Can IC be as fast and as efficient as UHPLC”?

Will the dream of fast ultra-high efficiency IC come true? This talk will discuss our long term efforts, tribulations, and triumphs in developing small particles in IC. The trials consist of evaluating non-polymeric substrates (1.8 - 3 [micro]m) for high efficiency IC. The tribulations consist of the packing process for small charged particles. The colloidal nature of these particles results in very unusual behavior in the packing process. The triumph part of this talk will consist of achieving high efficiency separations on IC columns packed with small particles.

Keywords: Ion Chromatography, Ion Exchange, Particle Size and Distribution, Separation Sciences
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 we 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 real-world analytical challenges.

Keywords: Chromatography, Environmental Analysis, Ion Chromatography, Ion Exchange

Application Code: Environmental

Methodology Code: Other (Specify)
Recently Advances in Ion Chromatography

**Characterizing the Mixed Cation Exchange-Reversed Phase Retention of Phosphorous Acid Coated Zirconia Columns**

Though ion chromatographic separations are principally performed on columns prepared with polymer supporting materials, for the obvious decrease in uncontrolled analyte interactions, other support materials can be used for these separations. One option, which provides exceptional physical and chemical robustness, is zirconia based support materials. Zirconia columns can withstand a greater range of buffer pH values, operating pressures, and temperatures, than silica and polymer based columns. This can allow for the use of unique mobile phases and separation conditions.

The modification of native zirconia particles to allow for controlled ion chromatographic separations has been shown to be achievable by employing the strong Lewis acid character of the zirconia to coat the particles with a strong Lewis base such as phosphoric acid and some of its derivatives. One such derivative that we have investigated is phosphorous acid (H\[sub\]3[/sub\]PO\[sub\]3[/sub\]), which revealed a surprising reversal in the selectivity for a series of benzyl trialkyl ammonium compounds, when compared to zirconia columns coated with phosphoric acid.

In studying the retentive properties of native zirconia columns coated with phosphorous acid, we have revealed that the phosphorus-hydrogen bond present on phosphorous acid provides a significant hydrophobic character to the stationary phase. This work will present our characterization of this modified zirconia surface as well as the influence of greater numbers of phosphorus-hydrogen bonds, through the modification of the zirconia with hypophosphorous acid (H\[sub\]3[/sub\]PO\[sub\]2[/sub\]).

**Keywords:** Characterization, Chromatography, Ion Chromatography, Ion Exchange

Application Code: Other (Specify)

Methodology Code: Liquid Chromatography
Recent Advances in Ion Chromatography

Application of Ion Chromatography in Flavor Science

The combination of activity-guided characterization and targeted quantitation of taste-active compounds with sensory experiments over the last years enabled the identification of various previously unknown food-borne tastants and first insights into the complex system generating the unique taste profiles of foods and beverages like cheese, meat, coffee, beer, and wine. While UHPLC-MS/MS has become the most important technique for quantitation of semi- and nonpolar analytes, the analysis of ionic and highly-polar compounds from complex food matrices is still a challenging task. The polar non-volatile sensometabolome of foods is mainly comprised of carbohydrates, minerals, polyols, organic and amino acids, which can be easily determined by means of ion chromatography coupled to various detection techniques. The presentation will outline the importance of ion chromatography as a complimentary separation technique to HPLC and the potential of recent developments such as capillary IC and High-Pressure IC for future applications in sensomics research providing data for the sensory-guided optimization of processing steps, selection of raw materials as well as quality control.

Keywords: Beverage, Capillary Ion Analysis, Food Science, Ion Chromatography

Application Code: Food Science

Methodology Code: Liquid Chromatography
Although methodology using HPLC/UHPLC is the workhorse of pharmaceutical analysis, ion chromatography (IC) has found itself a niche in pharmaceutical analysis for the determinations of both assay and impurities. The examples include IC applications for compounds that are not well separated on a reversed phase HPLC column, but can be separated using a cation or anion exchange column, and for the compounds that lack UV chromophore, but can be detected using conductivity or pulsed amperometric detection. The presentation will describe these types of IC applications for assay and impurities in pharmaceutical drug substance and drug products.
Rapid and sensitive chemical sensing using microfluidic device equipped with sterically-bulky three-dimensional gold nanostructure (Au3D) was demonstrated. Self-assembled noble metal nanoparticles have attracted many researchers since their nanostructure-depending unique plasmonic properties are available for sensing with surface enhanced Raman scattering (SERS). Au3D was fabricated by convective self-assembly known as "coffee-ring" phenomena of the mixed solution of colloidal gold and polystyrene latex particle. Particle concentration ratio of both particles, particle diameter, and infusion volume of the mixed solution were optimized. SERS measurements of a trace amount of 4,4'-bipyridine (4bpy) in aqueous solution were performed using Au3D in batch and flow format. PDMS flow module was fabricated by replica molding technique of Sylgard 184 with SU-8 as mold. Typical spectrum of 4bpy with enhanced peaks were immediately observed after dropping (batch) or injecting (flow). While detection limit was about 10 nM 4bpy in batch measurement, detection of further enhanced spectrum of 1 nM 4bpy was accomplished in flow measurement. Estimated enhancement factors were $6.7 \times 10^6$ in batch and $4.0 \times 10^7$ in flow. The enhancement factor in flow measurement is almost 6 times larger than that in batch measurement. This increment was considered to be derived from the number of molecules which accumulated on Au3D. Compared to two dimensional SERS substrate, the 37-fold large area of Au3D was also effective.

Thus, we confirmed that Au3D was available for convenient SERS optofluidic measurement.


**Keywords:** Lab-on-a-Chip/Microfluidics, Nanotechnology, Surface Enhanced Raman

**Application Code:** General Interest

**Methodology Code:** Microfluidics/Lab-on-a-Chip
Microfluidics: Novel Approaches

Microfluidic Sample Preparation for Liquid Characterization by XRF

The integration of microfluidic chips with micro x-ray fluorescence spectrometry (MXRF) offers a new approach for characterization of liquids. In the course of devising a sample preparation approach for actinide solutions, we have developed a microfluidic sampling methodology. The suitability (e.g., chemical resistance and optical transparency) of several common materials was assessed and a device was constructed using Kapton, polycarbonate, and silicone. In this feasibility study, a prototype microfluidic “chip” intended to serve as liquid sampling system for MXRF was designed and evaluated. Performance of the microfluidic sampling devices was evaluated using MXRF and high resolution x-ray (hiRX), a monochromatic wavelength dispersive x-ray fluorescence (MWDXRF) method which provides highly selective and sensitive detection of elements. The microfluidic chip was designed with a sample chamber 1 mm in diameter and contains approximately 1 [micro]L of sample when filled with a pipette. The performance of the devices was assessed with aqueous strontium solutions and synthetic spent fuel matrix. Between-chip and within-chip repeatability and linearity of response were studied. Preliminary limit of detection is 10 ng/[micro]L. This work demonstrates the applicability of microfluidic sample preparation to liquid characterization using MXRF.

The authors acknowledge the support of the U.S. Department of Energy through the Next Generation Safeguards Initiative (NGSI), Office of Nonproliferation and International Security (NIS), National Nuclear Security Administration (NNSA). Los Alamos National Laboratory is operated by the Los Alamos National Security, LLC for the National Nuclear Security Administration of the U.S. Department of Energy under contract DE-AC52-06NA25396.

Keywords: Lab-on-a-Chip/Microfluidics, Nuclear Analytical Applications, Sample Preparation, X-ray Fluorescence
Application Code: Nuclear
Methodology Code: Microfluidics/Lab-on-a-Chip
The extension of proteomic and metabolomic analyses to the single cell level is an exciting prospect for biological research, as the intercellular heterogeneity that is currently obscured by population-level analyses can be resolved by making chemical measurements one cell at a time. Mass spectrometry (MS)-based single cell analyses require highly sensitive detection as well as essentially lossless sample preparation. We have developed a microfluidic workflow for trapping, washing, lysing and delivering the contents of single cells to an integrated electrospray emitter in lossless fashion. Lysis is detergent-free to ensure MS compatibility and takes place within sealed compartments based on multilayer soft lithography to avoid sample losses. Following lysis, the cellular contents must be transported to the electrospray source for ionization and analysis. To avoid dispersion and dilution during transport, the contents of each cell are transported into aqueous droplets surrounded by an immiscible oil. In contrast to conventional droplet-based microfluidic platforms that generate droplets sequentially, the contents from the entire array of cell traps are loaded into droplets simultaneously. The droplets are then transported to the electrospray source for analysis. The integrated platform we present here constitutes a powerful tool for the label-free, high throughput analysis of proteins and metabolites from trace biological samples, while the simultaneous generation of aqueous droplets or plugs more generally enables parallel sample preparation to be combined with fixed point detection.

Keywords: Electrospray, Lab-on-a-Chip/Microfluidics, Mass Spectrometry, Proteomics
Application Code: Genomics, Proteomics and Other ‘Oomics
Methodology Code: Microfluidics/Lab-on-a-Chip
Recently, we introduced a new technique, namely bare narrow-capillary hydrodynamic chromatography (BaNC-HDC), for DNA separations in free solutions [1]. This technique was capable of sizing a wide range of DNA fragments in a single run, but precise sample injections were challenging. Latterly, we developed an on-chip injector to couple an electro-osmotic pump with BaNC-HDC for precisely loading samples at picoliter level [2]. However, injection and separation were still two isolate steps, which not only brought about complicated operations but also made continuous analysis impossible. Herein, we are proposing a new injection technique by incorporating a commercial injector and a microfabricated chip-splitter with BaNC-HDC. This incorporation allows introducing samples at sub-picoliter level. More importantly, injections can be conducted while separations are being performed, which makes flow injection analysis feasible and, as a result, greatly improves analysis throughput of BaNC-HDC. With the system proposed in this work, the 1-kb plus DNA ladders are baseline-resolved at the throughput of 14 assays/h while only a few molecules are required for each assay. Additionally, reproducibility and reliability of BaNC-HDC are considerably improved, and BaNC-HDC is expected to become a promising alternative technique to pulsed field gel electrophoresis.

Our research group is interested in quantitatively measuring rapid interactions associated with dopamine and other CNS neurotransmitters in vivo and in vitro by fast-scan cyclic voltammetry (FSCV) at carbon-fiber microelectrodes. In obtaining these measurements, microfluidics provides several advantages such as low sample consumption, high analysis throughput, and the ability to integrate multiple detection methods, such as fluorescence and amperometry. The most commonly used electrochemical detection scheme for microfluidics is constant potential amperometry; however, it has the disadvantage of poor selectivity compared to FSCV. Therefore, fabricating a microfluidic flow cell with an embedded carbon-fiber microelectrode for the application of FSCV provides the possibility of improved analyte selectivity while also being able to integrate other detection techniques. In this work, we fabricated a device consisting of two layers of polydimethylsiloxane (PDMS) with a fused-silica capillary sampling probe and an embedded working carbon-fiber microelectrode. Sample and buffer solutions are introduced into the microfluidic flow channel by utilizing a sequential injection technique and gravity feed. The carbon-fiber, embedded on the bottom layer of PDMS, is perpendicularly positioned to the flow channel of the top layer. A faradaic current is measured as sample travels above the electrode. Our studies indicate that this device provides good limits of detection of several biogenic amines, including dopamine and serotonin. In the future, this technique should allow for further studies of neurotransmitter release from brain tissues maintained on the microfluidic chip.

Keywords: Electrochemistry, Lab-on-a-Chip/Microfluidics, Neurochemistry, Voltammetry
Application Code: Neurochemistry
Methodology Code: Microfluidics/Lab-on-a-Chip
Surface modified, silicon, nanopillar structures continue to draw attention due in part to their unique optical properties and efficient chemical separation capabilities. To create separation media, dense pillar arrays with diameters in the 1-3 micron range and aspect ratios of approximately 20 are fabricated by photolithography with reactive-ion etching (RIE). This talk will focus on separation arrays used in an open planar platform driven by capillary action. The arrays have precisely controlled nano- and micro-scale architectures (e.g., independent control of pillar diameter and gap) and thus offer the potential for substantial improvements in separation efficiency and permeability over traditional separation media. Plate heights less than 2 micron are achieved due in part to capillary action-driven flow that is uniquely rapid and thus mitigates issues with diffusional broadening. The superhydrophobic property of the systems facilitate sample introduction. Electron beam lithography and RIE is used to create individual or small arrays of pillars in the 80-150 nm diameter range. With careful control of morphology, the unmodified pillars are effective in harvesting light and produce modest surrounding fields that are large enough to demonstrate non-plasmonic SERS and facilitate surface enhanced fluorescence (SEF) measurements. We demonstrate enhancements comparable to those achieved using plasmonic SEF structures without the limitations of the metal-based substrates. Experiments will be presented which explore these structures for applications in SEF bioassays. This research is supported in part by the National Science Foundation under Grant CHE-1144947 with the University of Tennessee and a portion was conducted at Oak Ridge National Laboratory.
Use of lab on a chip devices is constantly increasing, especially with the incorporation of sensors in tandem with micro- and nanofluidics. When military training facilities fire artillery and ammunition, heavy metals and other chemicals are given a direct route to contaminating ground water, affecting nearby communities. Consuming this contaminated water can potentially cause serious diseases affecting the circulatory, nervous, and immune systems. Sensors for micro- and nanofluidic devices have been developed for detection of heavy metals and other potentially hazardous chemicals in ground water and biological samples. Throughout experiments using this technology, biofilms have the potential to form on the sensors as well as the walls of these fluidic devices, leading to inaccurate readings of analyte concentration. Proteins can be one of the leading components in biofilm formation, making this a key area of research to find potential causes of biofouling under electrokinetic flow. Experimental variables responsible for biofouling under such electrokinetic environments include injection voltage, injection time, pH, protein concentration, and buffer concentration. Experiments looking into the effects of these variables are carried out in nanofluidic channels molded in a h-PDMS/PDMS mixture for fluorescence measurements using human fibrinogen tagged with Alexa Fluor 488 as a model protein.
A variety of spectroscopic and electrochemical techniques have been employed for real-time monitoring and detection in microfluidic devices. However, infrared (IR) spectroscopy has seen little implementation in the microfluidics field due to the IR-absorbing nature of commonly used microfluidic substrates. Real-time IR detection for microfluidic systems would be valuable for a large range of research interests. Some applications include the measuring of in-chip synthesis reaction kinetics, monitoring of enzyme-substrate interactions, and improved specificity or selectivity with (unlabeled) optical detection performed after a separation step. To achieve the goal of IR monitoring in microfluidic devices, we have developed several methods for constructing enclosed microfluidic channel networks. Channels are formed by either using a chemical etch with a polymer etching mask or by laser cutting a thin polymer layer that acts as an adhesive after heating. Both reversible and irreversible substrate sealing has been accomplished, and the methods employed have a range of fabrication complexities and equipment requirements. This presentation will discuss the microchip fabrication processes, specific approaches needed when using calcium fluoride substrates, and example application data illustrating the utility of real-time IR monitoring in microfluidic devices.
### Abstract Title
Potentiometric Scanning Ion Conductance Microscopy

### Abstract Text
Potentiometric scanning ion conductance microscopy (P-SICM) is a novel tool that is able to differentiate para and trans cellular pathways conductance in epithelial cell layers. This technique measures variation in potential generated at a surface with a dual-barrel probe design. One barrel is used as distance control by maintaining a constant ion current and the second barrel measures potential difference. Different from bulk measurement, P-SICM provides localized information by positioning pipet over different spots on a surface with a small probe-surface distance. Also, due to the precise feedback control given by SICM, P-SICM is able to utilize a much smaller pipet and probe-surface distance compared to traditional voltage scanning technique. However, the ion current for distance control and the small probe-surface distance make the system more complex. Therefore, to study the effect on the measured conductance from the unique features in SICM, a porous membrane with pores milled by Focused Ion Beam (FIB) is utilized as a simplified model for cell membranes. By measuring potential responses with different probe-surface distance, pore sizes, pipet current and pipet sizes, the detailed mechanism of P-SICM is understood. Data interpretation is more quantitative with exact geometry and property known in the system and proposed circuit model. By studying a FIB standard, the mechanism of the new technique P-SICM is understood providing guidance for more quantitative studies with biological samples.

### Keywords
- Analysis
- Bioanalytical
- Electrochemistry
- Nanotechnology

### Application Code
Bioanalytical

### Methodology Code
Electrochemistry
Trace metal pollution is a critical environmental and public health issue. Aqueous metal chemistry is sufficiently complex that metal mitigation systems cannot currently be used at their maximum efficiency. To more effectively treat metal contamination on-site, an instant diagnosis must be made. This can be achieved with an ultra fast, selective and portable detection method. Electrochemical techniques are easily portable; however, classical metal redox electrochemistry is diffusion driven and thus kinetically slow (minutes), not allowing real-time measurements. We recently described carbon fiber microelectrodes (CFMs) for Cu and Pb detection via fast scan cyclic voltammetry (FSCV) for ultra-fast (100 ms) metal detection. In this work, we explore the mechanisms responsible for fast redox chemistry on CFMs. We take a multi-analytical approach to describe the electrochemical, thermodynamic, kinetic and adsorption mechanism of fast metal voltammetry (FMV) for Cu2+. Electrochemically, we show that FMV is adsorption, not diffusion driven. We establish the nature of the redox couple responsible for the FMV signal via XPS and AFM analysis. Finally, we calculate the thermodynamic equilibrium constant for Cu2+ adsorption onto CFMs. This fundamental investigation into FMV mechanisms will allow us to further improve the sensitivity and selectivity of FMV for multi-metal analyses.

References

Keywords: Electrochemistry, Electrode Surfaces, Environmental Analysis, Imaging
Application Code: Environmental
Methodology Code: Electrochemistry
Ionic liquids (ILs) and polymeric ionic liquids (PILs) possess many characteristics, such as low vapor pressures and stability at high temperatures, which have resulted in success for their use in numerous applications including as sorbent coatings for solid phase microextractions (SPME). In addition to these characteristics and other factors such as extraction efficiency and selectivity, the ability to accurately control and reproduce the surface coating thickness remains an important aspect to consider when choosing a method for manufacturing of a SPME fiber. Here, an electrochemical method of deposition has been developed which allows for a well-controlled deposition of ionic liquids onto a conductive substrate for use as a SPME coating.

**Keywords:** Electrode Surfaces, Gas Chromatography, SPME, Voltammetry

**Application Code:** General Interest

**Methodology Code:** Electrochemistry
High temperature electrochemistry is an attractive technique as it offers the possibility of increasing analytical signals, investigating thermodynamic properties of different systems, and studying industrial processes that occur at elevated temperatures. Temperature pulse voltammetry (TPV) is realised with the use of a polycrystalline boron doped diamond electrode (pBDD) illuminated with near infrared laser irradiation. pBDD has been widely used as an electrode material due to its exceptional electrochemical properties; wide potential window, low background currents and chemical stability in extreme environments such as corrosive media. pBDD also possesses a very high thermal conductivity (＞600 W/mK at room temperature cf. 2200 W/mK for pure diamond) and a low specific heat capacity (0.50 J/kgK at room temperature). Thus diamond is an excellent heat spreader, and although it can be heated rapidly the energy can be quickly transferred to a heat sink (rather than the solution). Using 10 ms pulses from a 32 W 915 nm laser diode module focused (1 mm diameter spot) onto the diamond electrode temperature increases greater than 100 °C from room temperature could be achieved without changing the surface properties of the electrode. It should be noted that the laser illuminates the back side of the diamond, thus there is no interaction of the light with the solution/redox mediators.

Using a laser heated diamond electrode it is possible to significantly increase analytical signals for the different redox mediators investigated. The enhancement depends on the thermodynamic properties of the system under investigation as well as a number of other parameters, but the response to thermal pulses for different couples can be satisfactorily modelled. Furthermore, it is shown that the sensitivity of anodic stripping voltammetry to heavy metals in aqueous solution can be significantly enhanced using pulsed laser heating of the diamond electrode.
Voltammetry

Extra High Energy of Formation of Dianions Observed by Salt-Free Microelectrode Voltammetry

p-Benzquinone (BQ) and tetracyanoquinodimethan (TCNQ) which are reduced consecutively to mono-anions and dianions cannot be reduced voltammetrically up to the dianions without supporting electrolyte in acetonitrile solution. Voltamograms without supporting electrolyte at microelectrodes with the diameters less than 0.4 μm do not include IR-drop although the solution does not contain any ions. The conductance may be supported by ionic impurity, of which ionic resistivity is 2 kΩ. Formation of the dianions requires such large overpotential that acetonitrile may be decomposed reductively. In order to try to explain this overpotential, we derive analytical expressions for current-voltage curves of the sequential two-step electron transfer reactions in low concentration of electrolyte at a microelectrode from the Nernst-Planck equation and the Laplace equation. The overpotential cannot be explained in terms of the electric migration. With an increase in the conductivity of the solution by addition of electrolyte, the second reduction wave appears, involving the potential shift. The addition of the electrolyte does not influence the first reduction waves but varies the second one. The salt effect specific to the second wave is ascribed to electric interaction of the redox anion with the salt cation. The interaction generates non-stoichiometric complexes with salt cations, which can be observed by peak potentials with logarithmic variations of salt concentrations.

Keywords: Electrochemistry, Microelectrode, Voltammetry
Application Code: High-Throughput Chemical Analysis
Methodology Code: Electrochemistry
Voltammetry

Comparative Electrochemical Study of PANI/PSS and PANI-5%MWNT/PSS Films Obtained by Layer-by-Layer (LBL) Deposition onto ITO Substrates

Composites made with Polyaniline (PANI) and Carbon nanotubes (CNT) presents some advantages such as the increase of sensitivity, electrochemical capacitance and charge transfer. This work presents a comparative electrochemical study of layer-by-layer films (LBL) of PANI and PANI composite with five percent of functionalized multiwall carbon nanotubes (PANI-5%MWCNT). The PANI-5%MWNT composites were prepared by the adding MWNT to the monomer solution before synthesis initiation. The polymerization of PANI and its composite were made by the addition of ammonium persulfate solution onto the monomer solution. The LBL films were prepared by the intercalation of PANI or PANI-5%MWNT composites with Polystyrene Sulfonated (PSS) and were deposited onto ITO (Indium Tin Oxide) substrates. The analyses were performed by Cyclic Voltammetry technique. Figures 1 and 2 show the voltammograms obtained from the ITO electrodes modified with 10 bilayers of PANIPSS and PANI-5%MWNTPSS. It was observed that the PANI-5%MWNT film presents the increase of peak currents. All the voltammograms presented the PANI voltammetric behaviour.

References

Keywords: Electrochemistry, Electrodes, Nanotechnology, Voltammetry

Application Code: Nanotechnology
Methodology Code: Electrochemistry
The steady-state current at microelectrode $[i]a[/i]$ in radius is expressed by the average current density, $[i]j[/i]$ = $(4/[\pi'])[i]FcD/a[/i]$. On the other hand, the voltammetric current density at a large electrode is enhanced with an increase in scan rates $[i]v[/i]$. When the expression for the current density at the microelectrode is set equal to the equation for the peak current density by linear sweep voltammetry $[i]j[/i]$ = $0.446[i]Fc[/i]([i]DvF/RT[/i])^[sup]1/2[/sup]$, the relation between the scan rate and the radius is expressed by $[i]v[/i]$ = $8.15[i]RTD/Fa[/i]^2$. Typical values of $v$ for $[i]a[/i]$ = 1 nm are 210 MV/s. Therefore the steady-state voltammetry at microelectrodes allows us to obtain such fast electron transfer kinetic data as not to be realized by the conventional fast scan voltammetry. However, it is dangerous to consider simply equation from the diffusion process, because the heterogeneous reaction occurs at electrode surfaces rather than in the diffusion layer. This question will be discussed by determination of rate constants of fast electron transfer reactions of hexachloroiridium, hexaammine ruthenium, aminoferrocene, tetracyanoquinodimethan, ferrocenecarboxylic acid and ferrocene. This work is motivated by the experimental feasibility of high current density at ultramicroelectrodes without effects of capacitive currents or solution resistance. No potential shift is observed even at electrodes 0.01 [micro]m in diameter for the six species. This fact implies that the heterogeneous rate constants are at least larger than 10 cm/s. The inconsistency between the two methods is ascribed partly to solution resistance and partly to the resistance relevant to the electric double layer at fast scan voltammetry. The contribution of the solution resistance is demonstrated from the facts that the peak currents vary linearly with the peak potentials, that the slope of the linearity is independent of kinds of redox species.

**Keywords:** Electrochemistry, Microelectrode, Nanotechnology, Voltammetry
**Application Code:** Nanotechnology
**Methodology Code:** Electrochemistry